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The Journal of Clinical Investigation

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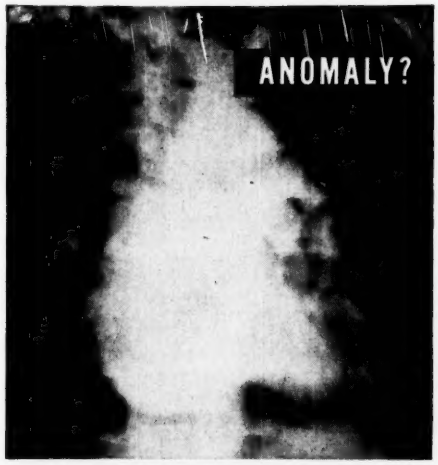

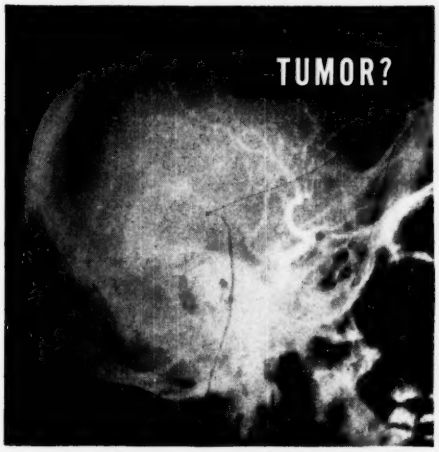

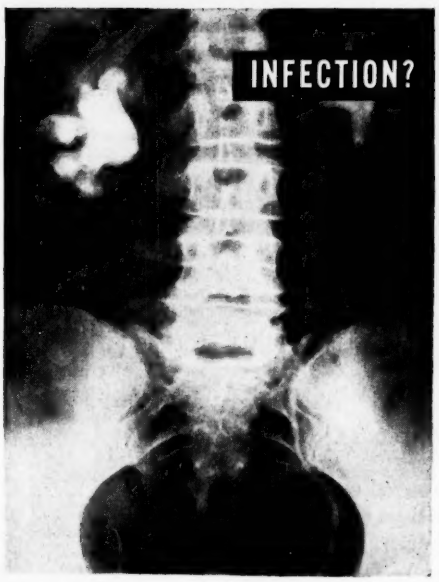

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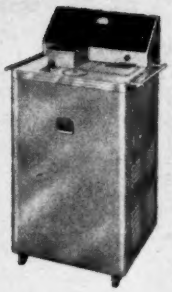

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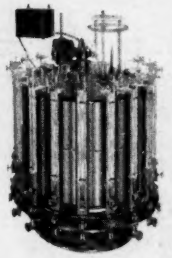

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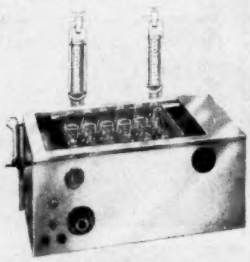

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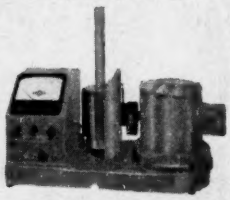

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fig. 1

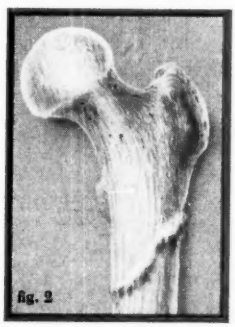
fig. 2

Femur, fracture, oblique, upper third.

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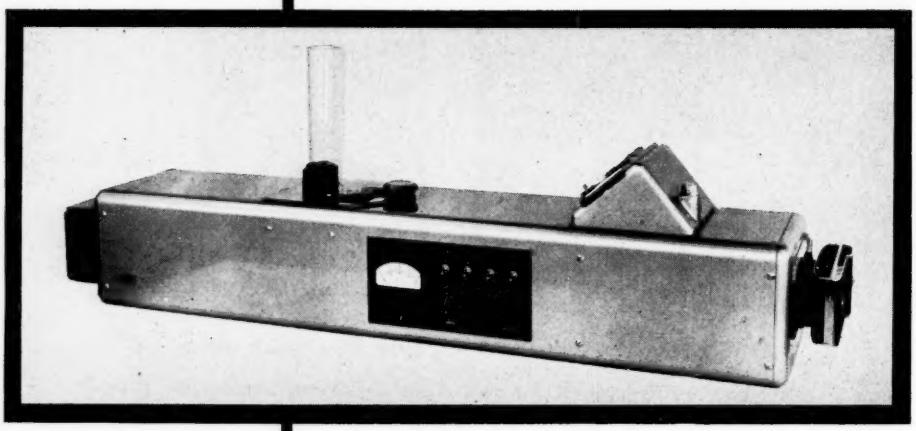

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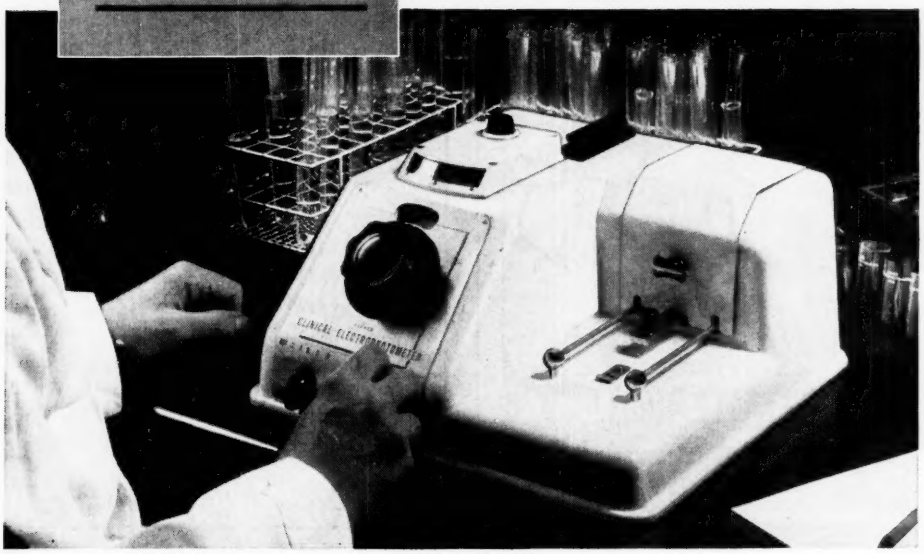

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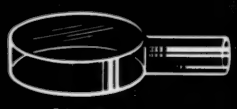

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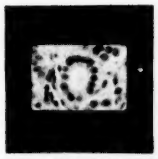

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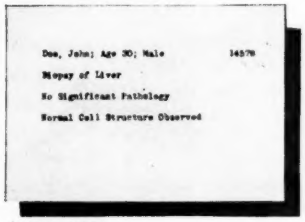

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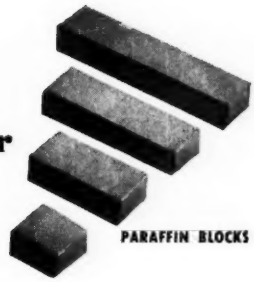

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THE RENAL EXCRETION OF INOSITOL BY NORMAL AND DIABETIC RATS[1]

By WILLIAM H. DAUGHADAY AND J. LARNER[2] WITH THE TECHNICAL ASSISTANCE OF ELLABETH HOUGHTON

(From the Departments of Medicine and Biological Chemistry, Washington University School of Medicine, St. Louis, Mo.)

(Submitted for publication January 22, 1954; accepted April 8, 1954)

Inositol is excreted in the urine of patients with diabetes mellitus in greatly increased amounts. Although this fact has been known since 1859, the cumbersome analytic procedures for inositol have prevented a thorough study of the mechanism of this abnormality (1). In an earlier publication from this laboratory a microbiologic assay was employed in clinical studies of the urinary excretion by normal and diabetic human beings (2). The increased amounts of inositol found in the urine of diabetic patients was related to glycosuria rather than to polyuria. The renal clearance of inositol was found to be much higher in diabetic than in non-diabetic patients and the administration of large amounts of glucose intravenously to non-diabetic patients raised the renal clearance of inositol to levels found in uncontrolled diabetic patients.

A comparable increase in the excretion of inositol in the urine of diabetic rats has been found. This report presents evidence that the inositol appearing in the urine of diabetic rats is largely of dietary origin. The mechanism of inositol excretion has been studied in the rat and observations are described which demonstrate that a mechanism exists for the tubular reabsorption of inositol which is inhibited by glucose and phlorizin but not by a saline diuresis. It is proposed that the renal tubular systems for the transport of glucose and inositol may be closely related.

METHODS

Adult male rats of the University of Illinois stock colony were subjected to subtotal pancreatectomy and were utilized in the experiments summarized in Table I. In all other experiments adult male Sprague-Dawley rats were used. Diabetes was induced by the intravenous administration of 45 to 60 mg. per Kg. of alloxan (Eastman). A period of one to three weeks was allowed for the recovery from the renal damage resulting from alloxan. The presence of diabetes was confirmed by measuring the level of glucose in the blood and urine.

The excretion of inositol by partially depancreatized and normal rats on low, medium, and high intakes of inositol has been measured (Table I*A*). Diet A was a purified diet[3] low in inositol (0.08 mg. per Gm.). Diet B was Purina Fox Chow which on analysis was found to contain 2.7 mg. of inositol per Gm. High inositol intake (Diet C) was obtained by feeding Fox Chow and adding inositol to the drinkng water. Because of the difference in fluid intake, 70 mg. of inositol per ml. of water was added for normal rats and 7 mg. of inositol per ml. for the partially depancreatized rats. After the animals had been eating the diet for six days the inositol excreted in the urine was measured. During the period of study rats were housed in metabolism cages and urine was collected under toluene, free of feces.

In the observations on alloxan diabetic rats (Table I*B*) the low inositol diet was fed for three days. Inositol was then added to the drinking water (100 mg. per ml. for normal rats and 50 mg. per ml. for diabetic rats).

[3] *Low Inositol Diet*

Casein	360 Gm.
Methionine	4 Gm.
Dextrin	1171 Gm.
Sucrose	300 Gm.
Salt Mixture (3)	80 Gm.
Cellu Flour	40 Gm.
Corn Oil	40 Gm.
Haliver Oil	1 Gm.
Choline HCl	4 Gm.
Thiamine Hydrochloride	40 mg.
Riboflavin	20 mg.
Nicotinic Acid	100 mg.
Pyridoxine Hydrochloride	50 mg.
Calc. Panthothenate	50 mg.
Para Aminobenzoic Acid	600 mg.
α Tocopherol	50 mg.
2-Methyl 1-4 Naphthaquinone	4 mg.
Biotin	200 mg.
Folic Acid	600 mg.

[1] This work was supported by a grant-in-aid from the Nutrition Research Foundation.

[2] This work was begun while holding a Postdoctorate Fellowship of the National Institutes of Health, and continued while a Fellow of the Life Insurance Medical Research Foundation. Present address: Division of Biochemistry, Noyes Chemical Laboratory, University of Illinois, Urbana.

TABLE I

Mean excretion of inositol by normal and partially depancreatized and alloxan diabetic rats

	Diet	Number of rats	Urine vol. *ml.*	Urine sugar *Gm./24 hrs.*	Inositol intake *mg./24 hrs.*	Urine inositol *mg./24 hrs.*
			A			
Normal rats	A	2	10	—	1.4	0.2
	B	2	8	—	41.0	0.5
	C	4	6	—	1,623.0	0.7
Depancreatized rats	A	4	231	19.1	3.3	2.5
	B	4	153	11.0	114.0	20.0
	C	6	169	15.6	1,426.0	400.0
Depancreatized rats after insulin treatment	A	4	69	2.5	2.3	0.5
	B	4	39	0.4	102.0	1.2
	C	4	31	0.2	2,965.0	38.0
Depancreatized rats after insulin plus salt diuresis	A*	2	174	0.2	2.6	0.45
	B†	2	177	0.2	89.0	1.7
			B			
Normal rats	A	6	—	—	1.5	0.2
	C	6	(Day 1)	—	474.0	12.0
	C	6	(Day 3)	—	570.0	2.0
	C	6	(Day 6)	—	822.0	20.0
Alloxan diabetic	A	5	—	18	1.6	5
	C	5	(Day 1)	15	1,032.0	209
	C	5	(Day 2)	16	1,091.0	228
	C	4	(Day 3)	17	1,196.0	293

* 10 per cent NaCl given by stomach tube. One rat received 21 ml. in seven doses, the other rat received 20 ml. in five doses.

† NaCl added to diet—5 per cent for one rat and 10 per cent for other rat.

The intestinal absorption of inositol was measured by the method of Cori (4). Prior to study the rats were fed the low inositol diet for three days to reduce the quantity of inositol in the gut. One hundred milligrams of inositol was given by gastric tube. After six hours the inositol remaining in the intestinal tract was recovered and measured.

For studies of renal clearance, rats were anesthetized with Nembutal® and a tracheotomy was performed. A polyethylene catheter was placed in either the femoral or external jugular vein and intravenous injections of priming and sustaining solutions were made with a simple syringe type infusion pump delivering about 8 ml. per hour. Heparin (0.2 mg.) was added to the initial loading solution. Urine was collected by catheter (5) for 30-minute periods following which the bladder was rinsed with 0.15 ml. of normal saline solution. At the midpoint of each period blood from the tail was collected in glass tubing (OD 4 mm.). After sealing with Pyseal the plasma was separated by centrifugation.

Inositol was measured by the method of Atkin (6) with the modifications previously described (2). The level of free inositol was determined in plasma after barium and zinc precipitation of proteins. Urine samples were not hydrolyzed routinely because it had been found in earlier experiments that inositol is excreted almost totally in the free form. Blood and urine sugar determinations were performed according to Nelson (7).

TABLE II

Intestinal absorption of inositol in normal and diabetic rats

	Number	Weight *Gm.*	Hours after inositol	Inositol: In gut *mg.*	Inositol: In urine *mg.*
Normal rats					
Control	3	120	—	1.8	—
Inositol (100 mg.)	3	137	0	104	—
Inositol (100 mg.)	6	129	6	73.7±2.63*	1.1
Diabetic rats					
Inositol	6	104	6	54.3±5.12*	10.5

* Standard error of mean. The difference in inositol in intestinal contents of normal and diabetic rats was significant, P = <.009.

TABLE III

Levels of blood inositol before and six hours after 100 mg. of inositol by stomach tube

	Number	Blood inositol: Initial *mg./100 ml.*	Blood inositol: 6 hours *mg./100 ml.*
Normal rats	5	2.9, S.E.±.20	3.0, S.E.±.52
Diabetic rats	6	2.3, S.E.±.32	5.4, S.E.±.74

A modification of the method of Folin and Wu (8) was used for the measurement of creatinine.

RESULTS

The influence of dietary intake on the excretion of inositol by normal and diabetic rats

Normal rats excreted little inositol even at the highest levels of inositol intake (Table IA). Partially depancreatized diabetic rats excreted far more inositol than did the control rats at all levels of intake tested. When insulin was given to diabetic rats in doses adequate to eliminate most of the glucose from the urine, the excretion of inositol was found to be nearly normal.

Inosituria has previously been attributed to polyuria of any cause (9). For this reason an experiment was done in which osmotic diuresis was induced by salt administration in diabetic rats receiving insulin. Although urine volume increased to levels comparable to that without insulin, the excretion of inositol remained low.

TABLE IV

Renal clearance of inositol in the rat

		Loading sol.		Sustaining sol.*		Creatinine			Inositol				Glucose	
	Period (30 min.)	Inositol mg.	Other mg.	Inositol %	Other	Plasma mg. %	Urine mg.	Clearance cc./hr.	Plasma mg. %	Urine mg.	Clearance cc./hr.	FFIR†	Blood mg. %	Urine mg.
Control	1	—	—	—		20	25.5	255	1	0.01	2	.99	—	—
No. 1	2	20	—	0.2		13.4	17.0	254	7	0.07	2	.99	—	—
Wt. 360 Gm.	3	—	—	1		17.6	12.3	140	14	1.4	20	.86	—	—
Control	1	—	—	—		19.5	23.0	234	0.7	0.01	2.9	.99	—	—
No. 2	2	20	—	0.2		27.7	26.0	188	17.5	0.6	6.9	.96	—	—
Wt. 453 Gm.	3	100	—	1		31.5	19.3	122	57.5	11	38.3	.69	—	—
Control	1	5	—	0.01		27.3	24.3	178	2.8	<.01	<1	.99	—	—
No. 3	2	10	—	0.05		46.5	32.2	138	7.8	.03	.8	.99	—	—
Wt. 325 Gm.	3	25	—	0.1		57.0	34.0	112	20.8	2.5	24	.79	—	—
Diabetic	1	5	—	0.01		23.0	28.4	247	3.3	0.5	30.3	.87	192	24
No. 1	2	15	—	0.05		30.3	32.9	181	12.7	3.1	48.7	.73	—	12
Wt. 262 Gm.	3	25	—	0.1		41.2	32.1	156	25.0	8.0	64.0	.59	—	8
Diabetic	1	5	—	0.01		18.5	23.3	252	3.9	0.5	26.2	.89	—	188
No. 2	2	15	—	0.05		24.9	24.8	199	11.4	3.2	56.2	.72	417	98
Wt. 364 Gm.	3	25	—	0.1		26.8	26.2	196	15.9	8.2	103	.47	—	62
Diabetic	1	5	—	0.01		57	11.8	42	6.3	0.8	24.5	.41	780	112
No. 3	2	15	—	0.05		92	18.8	41	15.9	2.7	33.9	.17	744	76
Wt. 266 Gm.	3	25	—	0.1		116	16.8	29	26.7	4.9	37	(−.27)	738	46
Glucose	1	5	600 mg. Gluc.	0.01	15% Gluc.	21	17.6	187	3.0	0.36	24	.84	516	238
No. 1	2	15	—	0.05	15% Gluc.	37.2	29.8	160	10.8	3.9	72	.55	554	306
Wt. 288 Gm.	3	25	—	0.1	15% Gluc.	43.5	26.2	120	21	10.9	104	.14	584	334
Glucose	1	5	900 mg. Gluc.	0.01	15% Gluc.	16.8	15.4	183	3.6	0.47	26	.86	655	380
No. 2	2	15	—	0.05	15% Gluc.	46.5	29.2	126	9.6	2.4	50	.66	850	284
Wt. 273 Gm.	3	25	—	0.1	15% Gluc.	52.2	28.2	108	17.2	7.7	90	.17	910	364
Glucose	1	5	600 mg. Gluc.	0.01	10% Gluc.	18.0	15.4	171	4.2	0.35	16.8	.90	442	205
No. 3	2	20	—	0.05	10% Gluc.	37.8	31.0	164	13.8	3.3	48.4	.70	468	185
Wt. 303 Gm.	3	40	—	0.1	10% Gluc.	43.5	34.6	159	27.8	15.2	109	.32	526	208
Phlorizin	1	15	—	0.1		17.3	19.8	229	14.1	4.3	61	.73	114	—
No. 1	2	—	187 mg. Phlor.	0.1		43.6	26.1	120	15.0	5.8	77	.36	131	64
Wt. 400 Gm.	3	—	—	0.1		49.7	30.3	122	14.7	4.4	60	.55	127	49
Phlorizin	1	15	—	0.1		26.2	22.9	175	23.8	1.9	16	.91	144	—
No. 2	2	—	187 mg. Phlor.	0.1		48.9	26.7	109	23.8	4.6	39	.65	148	60
Wt. 296 Gm.	3	—	—	0.1		67.2	36.2	108	20.0	4.1	41	.62	184	57

* Mannitol (3 per cent) was added to all hypotonic sustaining solutions. Mannitol was found not to affect inositol reabsorption. Creatinine (1 per cent) was present in all sustaining solutions.

† Fraction of filtered inositol reabsorbed $\left(1 - \frac{\text{Clearance of inositol}}{\text{Clearance of creatinine}}\right)$.

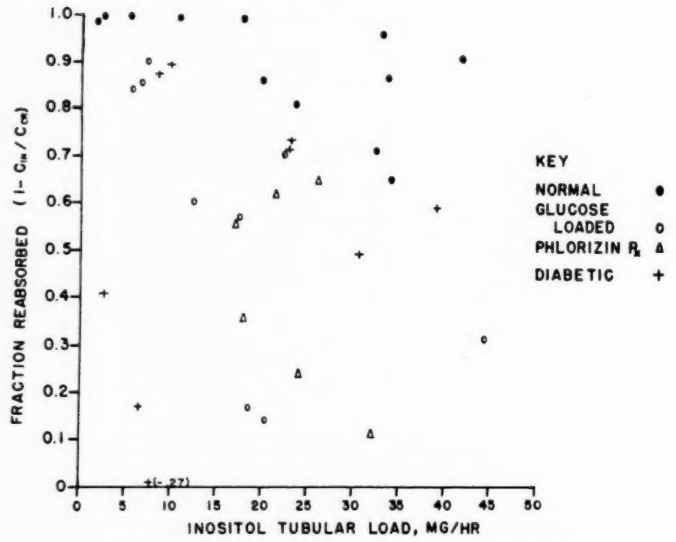

FIG. 1. A PLOT OF TUBULAR LOAD (PLASMA INOSITOL LEVEL X CREATININE CLEARANCE) AGAINST FRACTION OF THE TUBULAR LOAD REABSORBED

The results presented in Table I*B* indicate that the inosituria of alloxan diabetic rats is comparable to that occurring in partially depancreatized rats. Whereas normal rats excrete negligible amounts of large oral doses of inositol, alloxan diabetic rats excreted 20 to 25 per cent of that ingested.

Intestinal absorption of inositol

Little inositol was present in the gut prior to inositol administration and recovery was virtually complete immediately after inositol administration (Table II). Normal rats were found to have absorbed 26 per cent and the diabetic rats 46 per cent of the inositol which had been given six hours previously. This difference was found to be significant ($p = < .009$).

Because of the somewhat faster absorption of inositol from the gut by diabetic rats the concentration of inositol in the blood after acid hydrolysis has been determined before and six hours after the intragastric administration of 100 mg. of inositol. The mean level of blood inositol in fasted normal rats was slightly higher than that of diabetic rats but the difference is not significant (Table III). The blood inositol level after six hours in the diabetic rats was significantly higher than in the control group ($p = < .04$), despite the higher urinary excretion in the diabetic rats.

The renal clearance of inositol

At low levels of plasma inositol the renal clearance of inositol is very small in normal rats (Table IV). If the assumption is made that all the free plasma inositol is available for filtration the tubular load of inositol can be calculated. In Figure 1 the tubular load has been plotted against the fraction of filtered inositol reabsorbed ($1\text{-}C_{In}/C_{cr}$) which is indicated on the table as "FFIR." It is apparent that inositol reabsorption is virtually complete with tubular loads up to 17 mg. per hour. Thereafter increasing amounts of inositol appear in the urine. At tubular loads higher than presented in Figure 1, the inositol reabsorption increased despite inosituria and it has not been possible to define a definite Tm for inositol.

The inositol clearance of diabetic rats was at least ten times that of the non-diabetic rat. Glomerular filtration was impaired in the most severely diabetic rat (Diabetic No. 3). Whether this was due to alloxan injury or was a result of the severe diabetes could not be determined. The observation of a higher inositol clearance than creatinine clearance in Period 3 may represent an analytic error.

Glucose loading experiments were performed to distinguish the effects of hyperglycemia and

glycosuria *per se* from the other metabolic effects of insulin insufficiency. Consistent elevations of blood sugar were not obtained by intraperitoneal or subcutaneous administration of glucose. Hyperglycemia and glycosuria were induced by the intravenous infusion of glucose in three rats and the inositol and creatinine clearances were measured (Table IV). An increase in inositol clearance was observed under these conditions up to the range of that found in diabetes.

Phlorizin in a dose of 187 mg. intravenously was given to observe the effect on inositol clearance. Clearance measurements on two rats are presented in Table IV. In both rats there was a substantial reduction of the fraction of the inositol which was reabsorbed by the renal tubule.

DISCUSSION

The presence of increased amounts of inositol in the urine of rats with experimental diabetes is clearly established. Quantitatively the inosituria of diabetic rats is determined by inositol intake. The contention of Needham that "inosituria is associated with polyuria of whatever origin" (9) is not supported by the failure of a salt induced diuresis to re-establish the inosituria of diabetic rats receiving insulin.

In searching for other explanations for the increased excretion of inositol in diabetes mellitus the rate of gastrointestinal absorption and the mechanism of the renal excretion of inositol have been investigated. The intestinal absorption of inositol by normal rats is slower than that of the common hexoses and pentoses. Under the conditions examined an average of 3.4 mg. of inositol per 100 grams of rat was absorbed per hour as compared to the reported rate of 172 mg. of glucose per 100 grams of rat per hour (6). These findings agree with the more complete observations of Wiebelhaus, Betheil, and Lardy (10). The faster absorption of inositol from the gut of diabetic rats results in higher blood levels of inositol but probably contributes relatively little to the inosituria of such animals.

The studies of inositol clearance in the rat confirm the previous observation in man that inositol is virtually completely reabsorbed from the glomerular filtrate and the reabsorption of inositol is inhibited by glucosuria. A quantitative difference exists between the rat and the human being in respect to the degree of inhibition of inositol reabsorption by glucosuria. Inositol reabsorption was nearly totally abolished by glucose in the human; whereas, the inhibition in the rat proved incomplete and required a high glucose load for its demonstration. The relative specificity of the inhibition by glucose is suggested by the lack of effect of an osmotic diuresis produced by sodium chloride on inositol excretion. The addition of mannitol to hypotonic infusion solutions was also without effect on inositol excretion although diuresis occurred. The above data suggest that glucose and inositol may compete for the same tubular reabsorptive mechanism. The reabsorption of xylose has also been attributed to the glucose transport mechanism on the basis of similar evidence, namely the inhibition of xylose reabsorption by glucose and phlorizin (11). The interpretation of the action of phlorizin on inositol and xylose reabsorption is difficult. It is not possible to state to what extent the reabsorptive mechanism is inhibited by phlorizin directly and to what extent the inhibition is secondary to glycosuria.

SUMMARY

1. Depancreatinized and alloxan diabetic rats excrete much more inositol in their urine than do normal rats.

2. Whereas the inositol in the urine of normal rats is little influenced by dietary intake, that of diabetic rats is correlated directly with the amount of inositol ingested.

3. Diabetic rats absorbed inositol faster from the gut than did normal rats. The average inositol level in the blood of diabetic rats after oral inositol was found to be slightly higher than that of normal rats despite an increase in urinary excretion of inositol.

4. An active tubular reabsorption of inositol exists. The reabsorption of inositol is inhibited in diabetic rats, in rats with non-diabetic glycosuria and in phlorizin treated rats. An osmotic diuresis produced by sodium chloride did not result in inosituria comparable to that found in diabetes.

5. It is concluded that the inosituria of diabetes mellitus can be attributed to an increase in the renal clearance of inositol produced by glycosuria.

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CEREBRAL HEMODYNAMICS DURING CONTROLLED HYPOTENSION INDUCED BY THE CONTINUOUS INFUSION OF GANGLIONIC BLOCKING AGENTS (HEXAMETHONIUM, PENDIOMIDE AND ARFONAD) [1]

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Although the acute cerebral hemodynamic response to blood pressure elevation has been studied in normal man (1), there is very little information regarding the effect upon cerebral hemodynamics of reduction in blood pressure to hypotensive ranges. A moderate reduction in the systemic blood pressure appears to have very little effect on the cerebral circulation (2). However, when the blood pressure is markedly reduced one would anticipate that compensatory vasodilation of the cerebral vessels may be exceeded. Accordingly, the primary purpose of the current study was an estimation of the effect on the cerebral circulation of reduction in blood pressure produced by ganglionic blocking agents. An attempt was made to determine the critical level of reduction in arterial blood pressure beyond which further depression might produce cerebral ischemia and cerebral hypoxia. An estimation of this type is especially indicated because of the current use of controlled hypotension for some surgical procedures. The observations on the cerebral circulation in the current study were made on unanesthetized subjects in whom the blood pressure was reduced by continuous infusion of hexamethonium,[2] Pendiomide,[3] or Arfonad,[4] all of which are ganglionic blocking agents.

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[2] Supplied through the courtesy of Burroughs Wellcome & Co. as Hexameton, and Warner-Chilcott as Methium.

[3] Pentamethyl-diethyl-3-aza-pentane-1, 5 diammonium dibromide, supplied through the courtesy of Ciba Pharmaceutical Products as Pendiomide.

[4] D-3 4(1′, 3″-dibenzyl-2′ keto-immdizalido)-1,2,-trimethylene thiophanium d-camphor sulfonate, supplied through the courtesy of Hoffman-LaRoche as Arfonad.

METHODS

Observations on cerebral hemodynamics were made on 19 normal individuals using the nitrous oxide technique (3, 4) for estimating cerebral blood flow. The patients were divided into three groups. There were eight patients in group A who received hexamethonium, five patients in group B who received Pendiomide and six patients in group C who received Arfonad. The subjects were unanesthetized in order that obvious disturbances in cerebration could be detected should they occur when the blood pressure was reduced. All but one of the patients (No. 9) had normal arterial blood pressure at the time the studies were performed. The exception was a patient with labile hypertension whose blood pressure increased under stress, but was normal most of the time. Several additional patients showed a slight increase in systolic pressure at the time of the study, probably a result of the apprehension associated with the carrying out of the procedure. After suitable control observations (supine position) the blood pressure was reduced by administering one of the ganglionic blocking agents (hexamethonium,[2] Pendiomide[3] or Arfonad[4]). The cerebral blood flow studies were repeated after the desired reduction in arterial blood pressure had been obtained and the blood pressure had remained stable for 30 to 60 minutes. In patients who were particularly sensitive to any of the agents, an effort was made not to reduce the mean arterial blood pressure below 50 mm. Hg because of possible deleterious effects on the patient. The blood pressure was determined by simultaneous direct intra-arterial manometry and by auscultation and a mercury sphygmomanometer. The drugs were administered by continuous intravenous infusion using a concentration of hexamethonium and Pendiomide of 0.5 to 2 mg. per cc. of solution and of Arfonad of 4 mg. per cc. of solution in 5 per cent glucose in distilled water. The hexamethonium was given at the rate of 2 to 8 mg. per minute depending on the degree of reduction in arterial blood pressure that was attained. One patient who was relatively unresponsive to hexamethonium was given the drug at a rate of 8 mg. per minute for a total of 750 mg. Pendiomide was given at the rate of 3 to 6 mg. per minute. Usually a "floor" in the blood pressure was reached with both hexamethonium and Pendiomide and it was difficult to lower the pressure much farther. However, if the infusion was entirely discontinued the blood pressure usually rose 10 mm. Hg or

TABLE I

Cerebral hemodynamic response to blood pressure reduction with hexamethonium, Pendiomide or Arfonad *

Patient	Auscultatory blood pressure: Control Syst.	Control Diast.	Drug Syst.	Drug Diast.	Mean blood pressure: C	D	DT	Cerebral blood flow: C	D	DT	Cerebrovascular resistance: C	D	DT	Cerebral O_2 uptake: C	D	DT	Dose *mg./min.*	Total dose† *milligrams*
								Group A. Response to hexamethonium										
1	147	85	80	50	101	53	63	54	34	36	1.9	1.6	1.8	3.6	2.9	2.5	4	275
2	120	80	68	50	96	54	61	56	40	40	1.7	1.4	1.5	2.8	3.1	2.3	4	300
3	100	60	70	46	76	56	57	52	47	50	1.5	1.2	1.1	2.5	2.7	2.4	2	90
4	142	85	100	78	100	80	—	69	58	—	1.5	1.4	—	3.0	2.6	—	8	750
5	128	90	73	55	104	60	—	75	36	—	1.4	1.7	—	4.1	3.3	—	2	60
6	140	80	96	56	98	66	—	50	38	—	2.0	1.7	—	2.6	2.4	—	4	245
7	140	100	100	60	111	66	—	51	36	—	2.2	1.8	—	2.9	2.7	—	3	275
8	145	90	90	54	108	61	—	52	44	—	2.1	1.4	—	4.1	3.7	—	3	80
Mean	133	84	85	56	99	62	60	57	42	42	1.8	1.5	1.5	3.2	2.9	2.4		
P Value						<0.01			<0.01			<0.05			<0.3			
Mean per cent‡ of control			64	68		63	67		73	78		87	85		93	82		
								Group B. Response to Pendiomide										
9	178	100	70	48	122	54	63	46	42	46	2.7	1.3	1.4	2.8	3.2	3.4	3	100
10	116	70	80	50	94	54	58	51	45	60	1.8	1.2	1.0	2.3	2.4	3.0	5	700
11	130	66	98	56	90	65	72	57	60	62	1.6	1.1	1.2	2.5	2.9	3.3	6	500
12	120	70	90	66	84	72	81	50	38	40	1.7	1.9	2.0	3.3	3.1	3.2	6	350
13	136	90	84	54	111	60	—	49	51	—	2.3	1.2	—	2.9	3.2	—	5	500
Mean	136	79	84	55	100	61	69	51	47	52	2.0	1.3	1.4	2.8	3.0	3.2		
P Value						<0.01			<0.4			<0.05			<0.4			
Mean per cent‡ of control			64	72		63	73		93	102		70	75		108	120		
								Group C. Response to Arfonad										
14	116	70	60	40	92	48	46	51	34	30	1.8	1.4	1.5	3.5	2.4	2.5	5	300
15	120	78	70	46	94	44	42	41	25	27	2.3	1.8	1.6	2.9	2.6	2.0	9	800
16	140	92	78	50	110	58	47	48	35	48	2.3	1.7	1.0	2.5	2.7	2.4	9	650
17	112	80	92	60	96	75	—	58	38	—	1.7	2.0	—	2.8	2.9	—	21	1,500
18	130	86	92	64	106	66	—	73	56	—	1.5	1.2	—	4.1	4.1	—	6	500
19	132	90	70	52	96	52	—	40	25	—	2.4	2.1	—	2.6	2.0	—	4	225
Mean	125	83	77	52	99	57	45	52	36	35	2.0	1.7	1.4	3.1	2.8	2.3		
P Value						<0.01			<0.05			<0.2			<0.50			
Mean per cent‡ of control			62	63		58	46		68	75		86	65		91	79		
Mean Value, Groups A, B, and C	131	82	82	54	99	60	59	54	41	44§	1.9	1.5	1.4	3.0	2.9	2.7		
Mean per cent of control,‡ Groups A, B, C (19 patients)			63	67		61	63		77	87		82	75		96	96		
P Value (19 patients, Groups A, B, and C)						<0.01			<0.01	§		<0.01			<0.3			

* Mean blood pressure (mm. Hg) = direct arterial manometry; Cerebral blood flow = cc./100 Gm. brain/minute; Cerebral vascular resistance = mean blood pressure/cerebral blood flow; Cerebral O_2 Uptake = cc./100 Gm. brain/minute; C, D, and DT = See Table II for key to abbreviations.

† Includes all patients who received ganglionic blocking agents (hexamethonium, Pendiomide, or Arfonad).

‡ Mean value for per cent of control observations of individual studies.

§ Increase in cerebral blood flow due to head down tilt was not statistically significant, $P < 0.2$.

more within a few minutes. When the infusion was then started again the pressure could be depressed nearly to the previous levels, indicating that the rate of infusion exerted some effect (although small) on the degree of reduction in blood pressure after ganglionic blockade was fairly well established. Since the hypotensive response to Arfonad was more marked and the duration of action relatively short as compared to hexamethonium and Pendiomide, the rate of infusion was more closely related to the hypotensive response. Somewhat larger doses of this drug were used than in the cases of hexamethonium and Pendiomide. The rate of infusion of Arfonad varied from 4 to 21 mg. per minute. After maximum blood pressure reduction was attained with these agents the concentration of nitrous oxide in the jugular venous blood failed to approximate the concentration of nitrous oxide in the arterial blood within the usual 10 minutes. Therefore, it was necessary to continue the inhalation of nitrous oxide and sampling procedures for 20 minutes in order to obtain dependable determinations.

Following the observations in the horizontally supine position the infusion of the blocking agent was continued in some of the patients in each group and they were placed in a 30 degree head down tilted supine position. The manometer was adjusted to the level of the carotid artery, and was connected in such a way that it automatically corrected for the difference in elevation between the femoral artery and the carotid artery. After the arterial blood pressure had stabilized in this position, determinations of cerebral blood flow were again made. Seven of the patients who were not tilted were given a

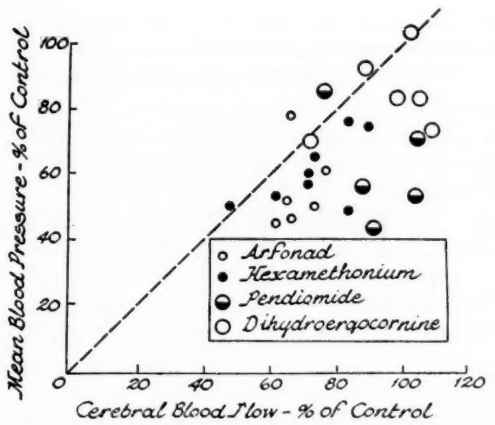

FIG. 1. CEREBRAL BLOOD FLOW FOLLOWING THE BLOCKING AGENTS (HEXAMETHONIUM, PENDIOMIDE, ARFONAD, AND DIHYDROERGOCORNINE) EXPRESSED IN PER CENT OF CONTROL AND PLOTTED AGAINST MEAN BLOOD PRESSURE EXPRESSED IN THE SAME MANNER

Cerebral blood flow is not depressed to the same degree as the mean blood pressure is and the pattern of response appears quite erratic. (Data on Dihydroergocornine taken from Hafkenschiel, Crumpton, and Moyer (2).)

vasopressor agent (nor-epinephrine or Aramine[5]) by continuous intravenous infusion in order to raise the blood pressure back to control levels or above. The rate of infusion of the blocking agent was not altered. Determinations of cerebral blood flow were then repeated. This offered additional information regarding the effect of reduction in blood pressure on cerebral hemodynamics as compared to any effect that the blocking agent might exert directly on the cerebral vessels.

RESULTS

There was a consistent reduction in mean blood pressure (P less than 0.01) in all patients. The rate of infusion was quite variable, and the total amount of drug employed if compared to the dog (5, 6) would have produced complete vagal ganglionic blockade with all three of the blocking agents. After the initial reduction in blood pressure was obtained, a "floor" was reached beyond which the pressure could not be lowered much farther regardless of the rate of infusion. If the initial rate of infusion was quite rapid the pressure was re-

[5] 'Aramine'; levo 1-(m-hydroxyphenyl)-2-amino-1- propanol.

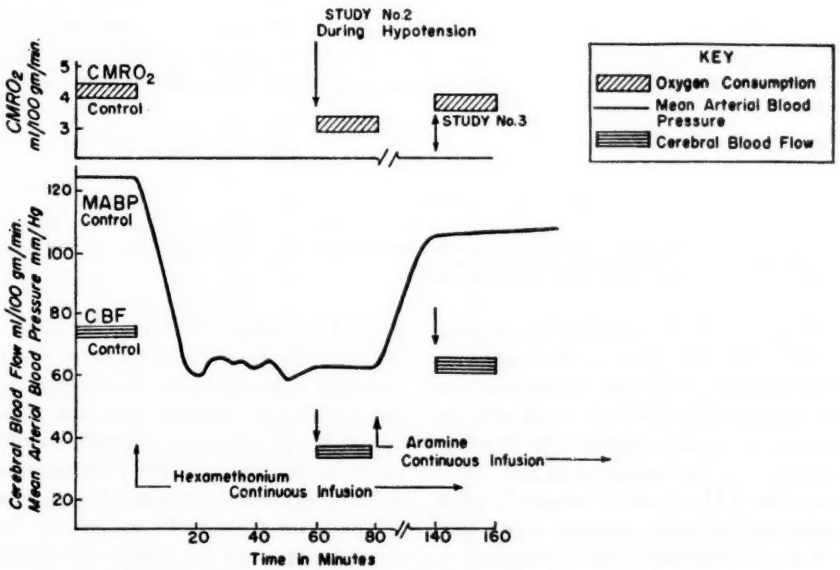

FIG. 2. THE EFFECT ON BLOOD PRESSURE (MABP) PLOTTED, ALONG WITH CEREBRAL OXYGEN CONSUMPTION ($CMRO_2$) AND CEREBRAL BLOOD-FLOW (CBF)

All are depressed following the establishment of ganglionic blockade with hexamethonium. However, when the blood pressure is elevated with an infusion of Aramine, cerebral blood flow and $CMRO_2$ increase towards the control value.

TABLE II

Effect of blood pressure reduction on cerebral blood oxygen and carbon dioxide *

Patient	Arterial O_2 *volume per cent*			Venous O_2 *volume per cent*			Cerebral arterial-venous O_2 difference *volume per cent*			Arterial CO_2 *volume per cent*			Venous CO_2 *volume per cent*			Arterial P CO_2		
	C	D	DT	C	D	DT	C	D	DT	C	D	DT	C	D	DT	C	D	DT
Group A. Response to hexamethonium																		
1	17.7	16.3	16.3	11.0	7.9	9.4	6.7	8.4	6.9	44.5	43.6	44.0	52.5	53.4	52.5	40	43	44
2	14.1	13.9	14.1	9.1	6.1	8.3	5.0	7.8	5.8	45.1	43.4	45.5	49.3	51.5	53.0	46	38	40
3	13.4	12.9	13.1	8.6	7.2	8.3	4.8	5.7	4.8	43.3	43.0	43.5	47.6	48.6	49.4	34	35	32
4	14.1	13.7	—	9.8	9.2	—	4.3	4.5	—	43.6	45.5	—	47.5	50.3	—	33	35	—
5	17.0	16.5	—	11.6	7.4	—	5.4	9.1	—	42.0	41.5	—	48.0	49.7	—	45	38	—
6	15.9	14.7	—	10.7	8.5	—	5.2	6.2	—	45.3	46.1	—	51.2	53.0	—	49	41	—
7	17.4	16.6	—	11.7	9.2	—	5.7	7.4	—	37.2	40.0	—	43.4	46.1	—	41	44	—
8	17.6	15.5	—	9.7	7.1	—	7.9	8.4	—	36.6	38.6	—	44.6	45.5	—	40	45	—
Mean	15.9	15.0	14.5	10.3	7.8	8.7	5.6	7.2	5.8	42.2	42.6	44.3	48.0	49.8	51.6	41	40	39
P Value		<0.3			<0.01			<0.05			<0.5			<0.3			<0.5	
Mean per cent of control†		95	97		76	91		129	106		101	100		104	104		98	97
Group B. Response to Pendiomide																		
9	15.9	14.3	15.0	9.7	6.6	7.7	6.2	7.7	7.3	47.9	48.4	48.4	53.0	53.9	55.3	43	42	39
10	18.3	17.9	17.1	13.8	12.6	12.1	4.5	5.3	5.0	41.1	42.3	42.0	45.6	47.1	46.8	43	45	45
11	14.4	14.2	13.6	10.1	9.4	8.2	4.3	4.8	5.4	44.6	46.1	45.5	49.4	50.2	50.0	33	33	33
12	18.2	18.2	17.7	11.7	10.1	9.6	6.5	8.1	8.1	38.1	38.4	37.1	44.3	45.0	45.5	38	39	39
13	15.1	13.4	—	9.1	7.2	—	6.0	6.2	—	41.2	42.5	—	46.9	49.1	—	42	44	—
Mean	16.4	15.6	15.9	10.9	9.2	9.4	5.5	6.4	6.5	42.6	43.5	43.3	47.8	49.1	49.4	40	41	39
P Value		<0.5			<0.3			<0.3			—			<0.5			—	
Mean per cent of control†		95	95		84	83		116	120		102	101		103	103		102	100
Group C. Response to Arfonad																		
14	13.8	12.1	12.6	7.0	5.1	4.4	6.8	7.0	8.2	46.9	49.3	44.5	52.6	55.1	52.0	38	41	36
15	16.2	15.7	15.7	9.2	5.6	8.4	7.0	10.2	7.3	43.6	43.1	45.4	49.5	52.9	53.9	35	35	36
16	14.1	15.9	14.5	8.9	8.2	9.4	5.2	7.7	5.1	45.2	40.0	46.4	50.0	47.6	52.5	33	28	34
17	16.4	18.6	—	11.6	10.9	—	4.8	7.7	—	42.3	43.6	—	46.6	51.3	—	41	49	—
18	15.9	16.4	—	10.3	9.1	—	5.6	7.3	—	46.4	51.6	—	52.7	58.1	—	42	48	—
19	20.0	18.3	—	13.6	10.3	—	6.4	8.0	—	41.3	43.8	—	47.0	51.2	—	43	47	—
Mean	16.1	16.2	14.3	10.1	8.2	7.4	6.0	8.0	6.9	44.3	45.2	45.4	49.7	52.7	52.8	39	41	35
P Value		>0.5			<0.20			<0.01			>0.5			<0.2			<0.5	
Mean per cent of control†		101	97		81	87		135	108		102	101		106	104		106	100
Mean Value, Groups A, B, and C	16.1	15.5	15.0	10.4	8.3	8.6	5.7	7.2	6.4	43.0	43.7	44.2	48.5	50.5	51.1	40	41	38
Mean per cent of control,† Groups A, B, and C		97	96		80	86		127	112		102	101		104	104		102	99
P Value (19 patients), Groups A, B, and C		<0.4			<0.01			<0.01			—			<0.10			—	

* C = Control; D = After blood pressure reduction with ganglionic blocking agent; DT = Thirty degree head down tilt during continuous infusion of blocking agent; P CO_2 = Partial pressure of carbon dioxide.

† Mean value for per cent of control observations of individual studies.

duced farther and could be maintained at a lower level than if the drug was given more slowly during the initial infusion. This type of response was particularly marked with Arfonad. Although the blood volume is not greatly altered with these hypotensive agents (7), the taking of as little as 15 to 20 cc. samples of blood would frequently cause a sharp reduction in blood pressure which persisted for 5 to 20 minutes. The explanation of this response to the taking of such small amounts of blood is not obvious to the authors.

When the mean blood pressure, determined by direct arterial mercury manometry, is compared to the auscultatory pressure there is usually a parallel reduction. However, at lower levels of pressure, the mean blood pressure which was determined manometrically occasionally approached the auscultatory diastolic pressure. This should be considered when only auscultatory observations on blood pressure are taken during surgical procedures employing controlled hypotension.

The blood pressure frequently did not increase significantly when the patient was placed in a 30 degree head down tilted position. In the three patients receiving Arfonad there was no increase after head down tilt perhaps a reflection of the greater degree of vasodilatation in these patients. The ganglionic blocking agents were administered

at the same rate in the tilted position as they were in the horizontal position, thus maintaining equivalent degrees of ganglionic blockade.

In the horizontally supine position, the cerebral blood flow was depressed (Groups A, B, & C) following the reduction in blood pressure in 17 out of 19 subjects, (P less than 0.01) but the change was usually not marked. Percentagewise the reduction in cerebral blood flow was not as marked as the reduction in mean blood pressure (Figure 1). Presumably, when the blood pressure is only slightly reduced initial vasodilatation is adequate (2). However, at lower levels of blood pressure, compensatory vasodilatation is insufficient to maintain the cerebral blood flow.

As the blood pressure was reduced, the oxygen was extracted more completely from the blood flowing through the brain. As a result, the jugular blood oxygen content was reduced (Table II) (P less than 0.01). This compensation (increased oxygen extraction) was not always adequate, and there was a general tendency for cerebral oxygen consumption ($CMRO_2$) to decrease. However, there were only four patients in whom the cerebral oxygen uptake per minute was reduced more than 0.5 cc. In all of these patients, the blood pressure was reduced to 60 mm. Hg or below. The reduction in $CMRO_2$ for 19 patients was not statistically significant (P less than 0.3).

There was no effect on the blood carbon dioxide except that the carbon dioxide content of jugular blood increased slightly (P less than 0.10). Partial pressure of carbon dioxide in arterial blood was not altered (Table II). The blood (arterial and venous) pH's were not altered significantly. For example, the mean arterial pH's for hexamethonium and Pendiomide during the control periods were 7.47 and 7.47, respectively, and were 7.50 and 7.46 after the drugs. The pH's were not altered by the head down tilt. The average venous pH's for hexamethonium and Pendiomide were 7.40 and 7.41, respectively, before the drug and 7.41 and 7.41 after it. After the tilt the pH's were 7.42 and 7.41, respectively. As with hexamethonium and Pendiomide, the arterial and venous blood pH's were not altered significantly by the administration of Arfonad.

Generally, the subjective manifestations for the

TABLE III

Side reactions to hexamethonium, Pendiomide and Arfonad and the effect on the respiratory rate, pulse rate, and hematocrit

Patient	Respiratory rate			Pulse rate			Hematocrit			Side effects during maximum hypotension
	C*	D*	DT*	C	D	DT	C	D	DT	
				Group A.	*Response to hexamethonium*					
1	15	17	16	72	90	92	43	43	43	Restless
2	22	20	24	101	89	92	45	41	40	Marked apprehension—improved on tilt
3	11	13	14	114	96	98	40	37	35	Felt chilly
4	18	18	—	88	90	—	38	36	—	Stuffy nose
5	23	19	—	112	130	—	50	47	—	Restless
6	18	16	—	80	78	—	30	31	—	None
7	14	17	—	80	76	—	49	48	—	Irregular respirations
8	24	22	—	88	68	—	41	40	—	None
				Group B.	*Response to Pendiomide*					
9	19	19	17	111	115	115	34	36	35	Irregular respirations, lethargy
10	28	25	27	89	93	89	37	37	38	Restless, cerebral symptoms, lethargy—improved on tilt
11	17	18	19	76	96	88	40	40	40	None
12	20	18	19	84	82	80	38	38	37	None
13	21	19	—	117	106	—	45	40	—	None
				Group C.	*Response to Arfonad*					
14	21	20	19	84	91	92	43	44	42	Dizzy, disoriented, nausea
15	20	21	21	81	90	85	45	42	43	Restless, vomited
16	22	21	20	84	87	81	41	41	41	None
17	18	16	18	59	66	67	50	50	50	None
18	21	21	—	84	85	—	45	44	—	Restless
19	22	20	—	84	84	—	50	49	—	Dizzy, restless, nausea, confused

* C = Control observations.
D = After blood pressure reduction with blocking agent.
DT = Observation with thirty degree head down tilt while receiving blocking agent.

patients in this study more or less paralleled the observations on cerebral blood flow and cerebral oxygen consumption ($CMRO_2$). Signs of cerebral hypoxia (restlessness, dizziness, syncope, disorientation) were not significant except in some of the patients in whom the mean arterial blood pressure approached 55 mm. Hg or fell below this level. The mean arterial blood pressure was reduced to 48 and 52 mm. Hg, respectively in the two patients (numbers 14 and 19) who became disoriented. However, in another patient (number 15) the mean blood pressure was reduced to 44 mm. Hg without producing disorientation, but this patient became nauseated and vomited. It appears that when the mean pressure was reduced below 55 to 60 mm. Hg the ability of the cerebral circulation to adjust adequately for the prevention of hypoxia was unpredictable. The response to Arfonad (Tables I and II) was essentially the same as to hexamethonium and Pendiomide except that the degree of reduction in blood pressure with Arfonad was slightly greater than with the latter agents. Consequently signs of hypoxia were more frequently encountered when the blood pressure was reduced with Arfonad. Symptoms of hypoxia which were associated with the reduction in blood pressure improved when the head was tilted down despite the fact that the observations did not indicate a significant increase either in cerebral blood flow or in cerebral oxygen uptake.

The effect on cerebral hemodynamics of raising the blood pressure from hypotensive to normotensive levels with Aramine or nor-epinephrine is demonstrated in Table IV. The mean arterial blood pressure increases more than cerebrovascular resistance. As a result, cerebral blood flow increases. At the same time the $A–VO_2$ difference decreases and cerebral oxygen consumption increases slightly.

COMMENTS

It appears that normotensive patients are consistently able to prevent cerebral hypoxia by a combination of vasodilation and increased extraction of oxygen from the blood when the blood pressure is not reduced below a mean pressure of about 55 to 60 mm. Hg. A number of patients (but not all) will even be able to adjust to blood pressure levels below 55 to 60 mm. Hg but it is unpredictable whether or not the compensatory adjustments will be adequate to prevent hypoxia. It is obvious in Figure 1 that there is no direct relationship between cerebral blood flow and mean arterial blood pressure. Initially, vasodilatation is nearly complete as was seen with Dihydroergocornine (2) in Figure 1. However, as the blood pressure was reduced farther, cerebral blood flow was depressed. When the mean blood pressure fell below approximately 55 to 60 mm. Hg cerebral oxygen uptake was also depressed in some patients. Although maximum vasodilatation may have occurred in these patients, it would appear that the brain is able to extract oxygen more completely (when the cerebral arterial blood pressure is adequate)

TABLE IV

Observations on cerebral oxygen metabolism and cerebral hemodynamic response to blood pressure elevation with vasopressor agent following initial hypertension due to ganglionic blockade

Patient	Ganglionic blocking agent	Vasopressor agent	Mean blood pressure (*mm. Hg*)			Cerebral blood flow*			Cerebro-vascular resistance*			Cerebral oxygen consumption*			Arterial-venous oxygen*		
			C§	D§	V§	C	D	V	C	D	V	C	D	V	C	D	V
3	Hexamethonium	Norepinephrine	100	80	140	69	58	65	1.5	1.4	2.2	3.0	2.6	3.4	4.3	4.5	5.2
5	Hexamethonium	Aramine	104	61	105	75	36	65	1.4	1.7	1.6	4.1	3.3	4.0	5.4	9.1	6.1
6	Hexamethonium	Aramine	98	66	118	49	36	64	2.0	1.7	1.8	2.6	2.4	2.9	5.2	6.2	4.5
8	Hexamethonium	Aramine	108	61	120	52	44	56	2.1	1.4	2.1	4.1	3.7	3.8	7.9	8.4	6.7
13†	Pendiomide	Norepinephrine	111	66	110	49	51	53	2.3	1.2	2.1	2.9	3.2	3.0	6.0	6.2	5.7
17‡	Arfonad	Norepinephrine	96	75	105	58	38	51	1.7	2.0	2.1	2.8	2.9	3.2	4.8	7.7	6.3
19	Arfonad	Norepinephrine	96	52	120	40	25	36	2.4	2.1	3.3	2.6	2.0	2.4	6.4	8.0	6.6

* See Table IA for methods of measurement.

† When blood pressure increased from 110 to 145 the cerebral blood flow decreased to 38 and the arterial-venous oxygen increased to 3.4 volume per cent.

‡ When blood pressure increased from 105 to 155, the cerebral blood flow decreased to 49 and the arterial-venous oxygen increased to 6.1 to 6.3.

§ C—Control observations. D—Observations after blood pressure reduction with ganglionic blocking agent. V—Observations after blood pressure elevation with vasopressor agent.

from the blood flowing through it, as has been previously demonstrated under conditions of hyperventilation (8) and after the administration of Aminophylline (9). The failure of more complete extraction of oxygen in the current study may be due in part to inadequate perfusion of plasma and oxygen through the capillary walls at these reduced blood pressure levels.

The reduction in cerebral blood flow appears to be related to the degree of blood pressure reduction rather than to the blocking agent employed. Since Arfonad is the most potent of the ganglionic blocking agents used one can expect to approach or pass below this critical level more frequently with this agent than with hexamethonium or Pendiomide if maximum blood pressure reduction is employed. Henry, Gauer, Kety, and Kramer (10) concluded that under conditions of gravitational stress, unconsciousness was not lost until the mean arterial blood pressure approached 25 mm. Hg. This is probably related to their method for lowering the blood pressure (positive acceleration) and the conclusions are hardly applicable to the current study. Scheinberg and Stead (11) observed that when the mean cerebral arterial pressure was reduced from 84 to 55 mm. Hg by changing from the supine to the upright position, there was a significant reduction in cerebral blood flow. This is in essential agreement with the current observations.

Cerebral vasoconstrictor tone is probably not affected directly by blockade of the sympathetic ganglia because procaine blockade of the superior cervical ganglia (12, 13) fails to increase cerebral blood flow. Since cerebral blood flow is most likely regulated by autonomous regulatory mechanisms at the local tissue level, the depression in cerebral blood flow which occurs during hypotension is merely a reflection of inadequate hydrostatic pressure. In the current study, when a vasoconstrictor agent was administered and the blood pressure raised from hypotensive levels to normotensive ones, the cerebral blood flow increased. The increase in cerebral blood flow which occurs under these conditions is in direct contrast to the cerebrovascular response to these same pressor agents (14) when administered to normotensive individuals with a subsequent increase in blood pressure to hypertensive levels since under the latter circumstances cerebral blood flow is reduced. If the reduction in cerebral blood flow which also occurs following the administration of ganglionic blocking agents were not a result of the hypotension, then raising the pressure with an adrenergic drug would not be expected to increase cerebral blood flow, but might actually reduce it (14). If such a pressure-flow relationship is accepted, then the value of vasopressor agents in the treatment of normal volemic hypotensive states seems well substantiated in so far as cerebral blood flow is concerned. These observations would tend to invalidate the suggestion (1) that the use of vasoconstrictor agents such as norepinephrine might depress cerebral blood flow when used for the treatment of severe hypotensive states.

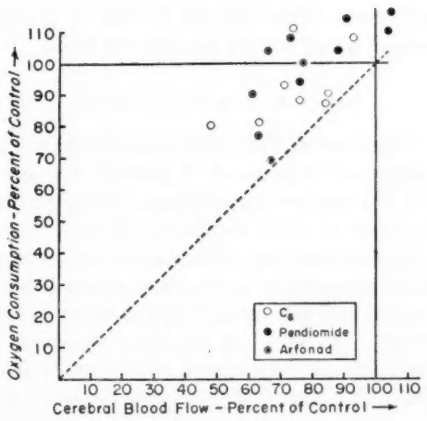

FIG. 3. CEREBRAL BLOOD FLOW EXPRESSED AS PER CENT OF CONTROL PLOTTED AGAINST OXYGEN CONSUMPTION EXPRESSED IN THE SAME MANNER

Cerebral blood flow is depressed in excess of cerebral oxygen consumption due to increased extraction of oxygen from the cerebral blood.

The failure of head down tilt to increase cerebral blood flow was a completely unexpected observation, and one for which the authors have no explanation. Some of the patients who demonstrated mental disturbances during hypotension were improved when the head was tilted down. This observation is not necessarily incompatible with the observations of a continued reduction in average cerebral blood flow per unit volume of brain since the improvement in cerebral function may merely represent improved circulation to the

cortical areas rather than an increase in the total amount of blood flowing through the brain.

SUMMARY AND CONCLUSIONS

1. Observations have been made on the cerebral hemodynamic response of 19 patients during controlled hypotension employing a continuous infusion of one of three ganglionic blocking agents; *i.e.*, hexamethonium, Pendiomide or Arfonad. Arfonad appeared to be the most potent hypotensive agent and the one with the shortest duration of action. A "floor" in the blood pressure was reached with all three agents, beyond which it was difficult if not impossible to reduce the blood pressure. It was possible to attain a maximum reduction in blood pressure by initially administering the blocking agent at a rapid rate. This was most apparent with administration of Arfonad.

2. After the blood pressure was reduced, cerebral blood flow decreased in 17 out of 19 subjects. The degree of reduction in cerebral blood flow was related to the degree of reduction of blood pressure rather than to any particular blocking agent. As the cerebral blood flow decreased, the oxygen was extracted more completely from the blood flowing through the brain, thus maintaining cerebral oxygen consumption. When the blood pressure was reduced below 55 to 60 mm. Hg this compensation was sometimes inadequate which resulted in a reduction in $CMRO_2$ (< 0.5 cc. per minute) and occasional symptoms of cerebral hypoxia. When the blood pressure was then raised to normotensive levels with a vasopressor agent, cerebral blood flow increased towards normal, thus indicating that the reduction in cerebral blood flow during the administration of the ganglionic blocking agent was probably due to the hypotension.

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SOME POSTURAL ADJUSTMENTS OF SALT AND WATER EXCRETION [1, 2]

By MORTON LEE PEARCE [3] AND ELLIOT V. NEWMAN WITH THE ASSISTANCE OF MARION R. BIRMINGHAM

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Among the acute adjustments of the normal human to orthostatic posture is a diminution in the rate of excretion of water and certain electrolytes (2, 3). This is associated with variable reductions of renal plasma flow and glomerular filtration rate (2, 4) and may actually occur without any appreciable change in the latter (3). The changes in renal hemodynamics are related to a fall in blood pressure and cardiac output which usually follows the assumption of upright posture (5, 6).

The causal sequence leading to orthostatic antidiuresis and antisaluresis is unknown. In acute experiments Pitressin administration leads to water retention without antisaluresis (7). That antisaluresis may occur without antidiuresis is less clear, although there is evidence that it may occur during alcohol diuresis (8, 9) which probably works through depression of the supraoptic posterior pituitary system. It also apparently occurs in venous congestion of the limbs in patients with diabetes insipidus (10). Orthostatic antisaluresis is reported to have been blocked by neck constriction (11) and legwrapping (12). Hypotonic expansion of the extracellular fluid volume in hydrated recumbent subjects results in increased sodium excretion (13). On the other hand, the administration of hyperoncotic albumin solutions results in diminished sodium excretion (14). In this instance antisaluresis is related to antidiuresis and is at least partially independent of the posterior pituitary since it occurs in patients with diabetes insipidus (15).

It was the purpose of the experiments reported here to investigate the effect on salt and water excretion of upright posture complicated as little as possible by vasomotor changes. An attempt was also made to determine whether the antidiuresis and the antisaluresis of standing are interdependent or controlled by separate mechanisms. To this end three sets of experiments were performed. In the first the effect of standing on salt and water excretion was measured. In the second an attempt was made to inhibit the secretion of Pitressin by administering alcohol to the subjects. In the third set of experiments legwrapping was employed to inhibit postural antisaluresis. The results obtained in the last two sets of experiments were compared with the first, using each subject as his own control.

METHODS

The subjects were normal adult laboratory personnel and medical students. No attempt was made to control the diet over the days preceding the experiments. The experiments were done in the morning with the subjects fasting. Upon arising in the morning the subjects emptied their bladders, noted the time and ingested 250 to 400 cc. of water. Upon arrival at the laboratory they again voided (U_1) and then rested supine. Voided urine was collected every 20 minutes and 150 cc. of water were ingested after each urine collection. After water diuresis had been established (one to two hours), the subjects were tilted to 60° from the horizontal position for one hour. To avoid marked shifts in fluid compartments and vasomotor effects the subjects were encouraged to shift their legs occasionally during this hour. Following the orthostatic period, urine collections were made for another hour with the subject supine. The experimental period was thus bracketed by two control periods. This was considered important because of known fluctuations in salt and water excretion during various hours of the day, and from day to day. The experiments were performed in a quiet room with only essential personnel entering and leaving the room. Smoking was interdicted because of the antidiuretic and vasomotor effects associated with it.

In the experiments in which alcohol was administered, 25 cc. of 95 per cent ethanol and 125 cc. of grapefruit juice were substituted for the 150 cc. of water ingested at the time of tilting and 20 minutes before it. This is

[1] A preliminary report appeared in abstract form (1).

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TABLE I

Control experiments

Subject	Control period preceding tilt					Maximum tilt effect					Last period of second control				
	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.
M. L. P.	121	13.0	.191	.156	.110	125	3.1	.063	.053	.029	152	13.3	.143	.101	.086
M. B.	119	13.2	.112	.092	.106	101	2.36	.040	.065	.066	111	16.0	.176	.138	.140
M. B.	107	14.1	.177	.226	.153	97	4.5	.082	.115	.052	108	14.2	.250	.195	.137
C. M.	111	11.8	.348	.339	.159	100	2.45	.105	.147	.078	109	11.0	.284	.276	.143
E. V. N.	83	13.0	.208	.180	.107	70	4.75	.045	.082	.064	81	10.2	.179	.144	.089
D. McC.	96	11.7	.102	.106	.117	87	6.6	.053	.076	.077	101	13.0	.147	.151	.139
M. Y.	96	12.3	.180	.226	.049	84	6.1	.090	.142	.020	83	6.7	.154	.181	.088

TABLE II

Experiments in which alcohol was given before tilt

Subject	Control period preceding tilt					Maximum tilt effect					Last period of second control				
	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.
M. L. P.	144	11.8	.200	.212	.254	111	10.7	.068	.058	.081	126	11.4	.125	.089	.091
M. B.	113	10.8	.121	.111	.070	101	10.5	.040	.052	.034	104	13.6	.101	.079	.134
C. M.	125	13.2	.371	.360	.138	119	7.2	.057	.073	.036	100	8.7	.124	.110	.044
D. McC.	87	11.6	.123	.131	.055	87	13.0	.065	.078	.019	84	2.75	.137	.143	.032
M. Y.	115	15.7	.210	.227	.047	111	11.8	.080	.107	.014	116	12.4	.152	.149	.088

TABLE III

Experiment in which alcohol was given but subject remained supine through usual tilt period

Subject	Control period preceding tilt					Maximum tilt effect					Last period of second control				
	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.
M. L. P.	148	13.5	.179	.166	.128	140	15.8	.126	.128	.052	130	5.8	.130	.105	.042

TABLE IV

Experiments in which legs were wrapped before tilt

Subject	Control period preceding tilt					Maximum tilt effect					Last period of second control				
	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.	Creatinine clearance	Urine ml./min.	Na mEq./min.	Cl mEq./min.	K mEq./min.
M. L. P.	137	13.6	.181	.177	.090	117	8.3	.142	.128	.073	127	13.6	.255	.172	.075
M. L. P.	137	11.5	.136	.141	.122	134	3.9	.086	.082	.057	131	11.6	.135	.104	.087
M. B.	134	19.1	.168	.164	.088	134	3.4	.144	.156	.067	142	14.8	.229	.160	.092
C. M.	120	13.9	.264	.340	.111	108	6.5	.216	.262	.076	115	10.1	.273	.332	.097
C. M.	109	11.5	.338	.274	.161	104	4.8	.216	.235	.141	98	10.6	.268	.244	.151
E. V. N.	106	13.7	.206	.219	.141	99	3.65	.080	.106	.077	101	10.2	.159	.184	.095
E. V. N.	107	12.7	.178	.203	.124	95	3.8	.098	.155	.095	104	9.8	.181	.179	.110
D. McC.	98	12.4	.040	.046	.027	87	9.4	.024	.059	.037	98	7.7	.063	.101	.052
M. Y.	98	15.0	.208	.266	.101	83	9.5	.082	.142	.101	92	12.2	.254	.235	.104

equivalent to about three whiskey highballs and produced a mild degree of inebriation in the subjects.

In the experiments in which legwrapping was employed the legs were wrapped tightly with three inch "Ace" bandages from the toes to the groin just before tilting. The bandages were removed as soon as the supine position was again assumed.

In order to avoid the effects of pain only one venipuncture was performed in most of the experiments. This is thought to be justified because changes in serum

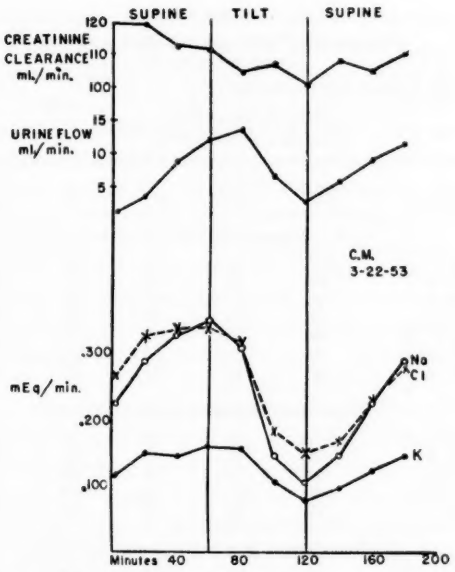

FIG. 1. THE EFFECT OF ASSUMING THE ORTHOSTATIC POSITION ON SALT AND WATER EXCRETION

concentrations were slight while the changes in urinary constituents were marked.

Creatinine determinations were performed on serum and urine immediately after the experiment by the method of Peters (16). Sodium and potassium were measured

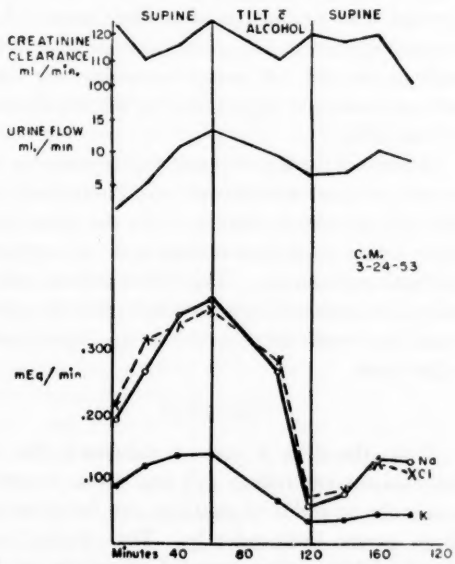

FIG. 2. THE EFFECT OF ALCOHOL ON THE POSTURAL ADJUSTMENTS OF SALT AND WATER EXCRETION

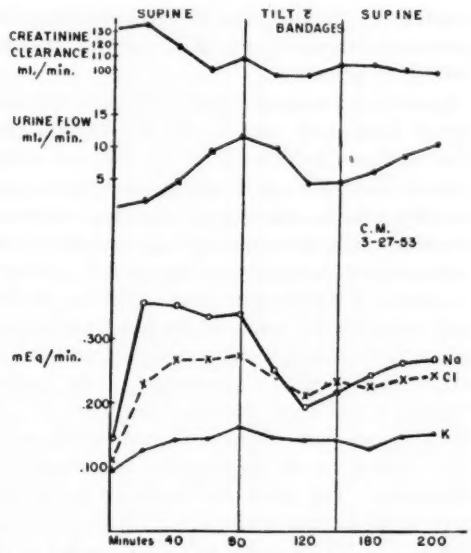

FIG. 3. THE EFFECT OF LEGWRAPPING ON THE POSTURAL ADJUSTMENTS OF SALT AND WATER EXCRETION

with a Beckman Model B. flame photometer. Chloride was measured by the potentiometric method of Sanderson (17).

RESULTS

Pulse and blood pressure were determined and remained fairly constant in all experiments. The means of the three groups were: Tilt alone; supine P 65, B.P. 117/75, standing P 70, B.P. 120/83. Alcohol experiments; supine P 61, B.P. 115/73,

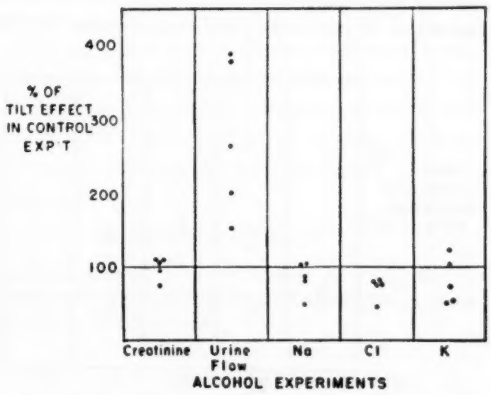

FIG. 4. THE EFFECT OF ALCOHOL EXPRESSED AS PERCENTAGE OF THE UNCOMPLICATED TILT EXPERIMENTS USING EACH SUBJECT AS HIS OWN CONTROL

standing P 78, B.P. 113/77. Legwrapping experiments; supine P 66, B.P. 121/78, standing P 70, B.P. 121/84.

In order to conserve space only one collection period from each control and the experimental hour is shown in Tables I to IV. The last collection of the two control periods and the experimental period in which maximum effect was noted are used. The three types of experiments on one of the subjects are shown in Figures 1–3. Table V is derived from the preceding tables by dividing each experimental result by the comparable figure in the first control so that each experimental result is expressed as a percentage of the control. Figures 4 and 5 are derived from Table V. In these figures each subject's tilt experiment served as a control for the legwrapping and alcohol experiments. The latter are expressed as percentages of the control study, *i.e.*, (Tilt/Supine) (alcohol) ÷ (Tilt/Supine) (Control study) × 100 per cent.

In most of the experiments there was a fall in creatinine clearance to about 90 per cent of the control values. In the uncomplicated tilt experiments there was usually a greater percentage fall in water excretion than there was in electrolyte excretion so that the urine was concentrated as compared with the control period. In most of the experiments the greatest electrolyte change was in sodium and the least in potassium; chloride effect was intermediate. When alcohol was administered salt excretion fell more than in the uncomplicated tilt experiments while water excretion remained at 90 per cent of the control value on the average with production of a dilute urine. In a single experiment in which alcohol was given without the 60° tilt, water excretion rose while salt excretion fell slightly during the second hour (Table III).

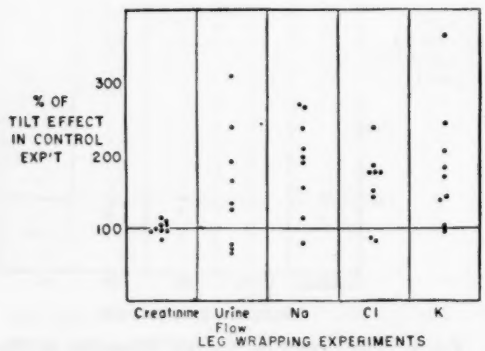

FIG. 5. THE EFFECT OF LEGWRAPPING EXPRESSED AS IN FIGURE 4.

TABLE V

Comparison of maximum tilt effect to control period preceding tilt

Each number represents the ratio:

$$\frac{\text{Value in tilt period}}{\text{Value in control period}} \times 100$$

Subject	Creatinine	Urine flow	Na	Cl	K
A. Experiments with tilt alone					
M. L. P.	103	24	33	34	26
M. B.	85	18	36	71	62
M. B.	91	32	46	51	34
C. M.	90	21	30	43	51
E. V. N.	84	36	22	45	60
D. McC.	91	56	52	72	66
M. Y.	88	49	50	63	41
B. Experiments in which alcohol was given					
M. L. P.	77	91	34	27	32
M. B.	90	97	33	47	49
C. M.	95	55	15	20	26
C. McC.	100	112	53	59	35
M. Y.	97	75	42	47	30
C. Experiments in which legs were wrapped					
M. L. P.	86	61	79	72	81
M. L. P.	98	34	63	58	47
M. B.	100	18	86	93	76
C. M.	90	47	82	77	68
C. M.	96	42	64	86	88
E. V. N.	93	27	39	48	55
E. V. N.	89	30	55	76	77
D. McC.	89	76	60	128	137
M. Y.	85	63	40	53	100

In some of the legwrapping experiments the expected postural antidiuresis was diminished, but this was not always the case. On the other hand, there was a consistent reduction of the expected postural antisaluresis. This effect is most noticeable when each subject's uncomplicated tilt experiment was used as control for the legwrapping experiment.

DISCUSSION

From the data it can be suggested that the mechanisms controlling salt and water excretion under the stimulus of standing can function to a large extent independently. That alcohol produced inhibition of an expected orthostatic antidiuresis suggests that this postural adaptation may

be under supraoptic posterior pituitary control, although it may have been due in part to a suppression of antidiuretic activity existing before the tilt. The result is the converse of water retention without salt retention produced acutely by the administration of Pitressin (7).

There are several possible explanations for the reduction in postural antisaluresis produced by legwrapping. Postural antisaluresis may be produced by a stretch of veins in the lower part of the body. The increase over the control orthostatic antisaluresis in the alcohol experiments is not in agreement with this, since the blood volume is, if anything, diminished by the subsequent dehydration. The collapse of veins in the upper part of the body is a possible explanation, but the antisaluresis produced by hyperoncotic albumin (13) is not in agreement. The latter expands the intravascular space at the expense of the interstitial fluid space. The antisaluresis may be mediated in part by the fairly consistent 10 per cent fall in creatinine clearance which occurred upon standing. However, it should be noted that the fall in creatinine clearance was approximately the same whether or not the legs were wrapped. Since the postural antisaluresis was diminished following legwrapping, the fall in glomerular filtration rate is not an adequate explanation for the entire postural effect on salt excretion.

A loss of interstitial fluid from some volume sensitive area is a reasonable explanation for postural antisaluresis. Both the legwrapping and alcohol experiments support this suggestion, as do the experiments of Strauss, Davis, Rosenbaum, and Rossmeisl (13) who administered hypotonic solutions which would in part expand the interstitial space. The antisaluresis produced by hyperoncotic albumin (14, 15), venous compression of the legs (18) and the superior vena cava syndrome (19) are probably caused by the mechanism responsible for postural salt retention. It is difficult to reconcile the observations of Lewis, Buie, Sevier, and Harrison (11) in which it was suggested that the volume sensitive locus is in the cranium because postural antisaluresis was inhibited by neck constriction. This observation does not fit with the observations on the superior cava syndrome (19) in which intracranial venous pressure is also high. Netravisesh (20) was unable to confirm the neck constriction experiments, and in a single experiment employing the control-tilt-control protocol, neck compression failed to block the expected postural antisaluresis, although the compression was enough to produce facial edema.

Partially independent posture-sensitive mechanisms for the control of salt and water excretion seem to be present. Normally they function together and altering one of them may change the response of the other. In the light of present knowledge the best explanation is that there is some locus sensitive to the volume of the interstitial space which initiates postural antidiuresis and antisaluresis. Postural antidiuresis may be mediated through the posterior pituitary. The pathway mediating postural antisaluresis is not clear at this time.

SUMMARY

In the normal human subject undergoing moderate water diuresis quiet standing results in a slight depression of creatinine clearance and a marked depression of sodium, chloride, potassium, and water excretion as compared with control measurements in the supine position. If the legs are wrapped with elastic bandages before the orthostatic position is assumed the depression of salt excretion is inhibited with varying effect on the antidiuresis. On the other hand, administration of alcohol inhibits the orthostatic antidiuresis and enhances the orthostatic inhibition of salt excretion.

It is suggested that there are separate postural adjustments of water and salt excretion, the former under diencephalic posterior pituitary control and the latter under the control of some mechanism sensitive to the distribution of interstitial fluid.

ACKNOWLEDGMENT

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A POSSIBLE FOURTH PLASMA THROMBOPLASTIN COMPONENT

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Recent studies have shown that the clinical and laboratory findings of hemophilia can be duplicated by at least two other blood coagulation disorders of related nature. In each case, laboratory studies have revealed a prolonged whole blood clotting time, reduced utilization of prothrombin in the course of clotting, and correction of the defect by the addition of small amounts of normal plasma. In none of these newly reported cases has a circulating anticoagulant been demonstrated, and all had normal amounts of fibrinogen, prothrombin, and both prothrombin conversion accelerators. On the basis of such findings these new conditions, like hemophilia, may be considered to involve a disturbance in intrinsic thromboplastin formation of the blood.

That the condition traditionally designated, "hemophilia" is not a disorder of uniform nature was first suggested in 1947 by the studies of Pavlovsky (1) wherein it was found that the blood of certain "hemophiliacs" improved clotting in others. In 1950, Koller, Krüsi, and Luchsinger (2) reported on a family of bleeders whose blood was likewise corrective against the coagulation defect of classical hemophiliacs, and for the first time recognized that a new disorder accounted for this phenomenon. The investigations of Aggeler, White and their co-workers (3–5) on a patient of this type established the existence of a new plasma thromboplastic factor. The new factor was found to contrast sharply with antihemophilic factor (AHF) by its presence in serum as well as plasma, its stability to storage at refrigerator temperature, its complete adsorption by barium sulfate, and its precipitation from plasma only at concentrations of ammonium sulfate greater than 40 per cent. Moreover, AHF is found mainly in Cohn's fraction I; the new factor is in fractions III and IV.

Reports of cases probably similar in nature soon followed. The name, "thromboplastin component (PTC) deficiency" was chosen for the disease by Aggeler and his co-workers (3). Biggs and associates (6) designated it "Christmas Disease" and Soulier and Larrieu (7) suggested the use of hemophilia A and B to differentiate the two disorders.

Recently, Rosenthal, Dreskin, and Rosenthal (8) described still another hemophilia-like condition which appears to be due to the deficiency of a third plasma thromboplastic factor. This third factor has been named "plasma thromboplastin antecedent (PTA)." The coagulation disorder in these patients is corrected by both hemophilic and PTC-deficient plasma, as well as by normal plasma and serum. PTA was found to be absent from the 0 to 20 per cent ammonium sulfate precipitate of normal plasma, but was present in the 20 to 33 per cent fraction. As in the case of PTC, PTA was absent from fraction I of Cohn. Although normal plasma which had been adsorbed with barium sulfate corrected clotting in PTA-deficient patients, a partially purified PTC preparation made from a barium sulfate eluate of normal plasma was also partially corrective.

The studies to be described in the present report suggest the existence of an additional new member of the plasma thromboplastin complex and a corresponding deficiency thereof, resulting in another hemophilia-like disorder.

Report of case: The patient, C. C., was a thirty-one year old white American male who had had frequent and severe epistaxes from the ages of seven to eighteen. During this period four dental extractions were each followed by consistent oozing from the gums lasting one to two months. At the age of eighteen the patient was in an airplane crash, and as a result of injuries required an amputation of the right leg below the knee. Ten days after this procedure the wound began to bleed, and permanent hemostasis was not obtained for four months. Four revisions of the stump were attempted in the succeeding six year period, and each of these procedures was accompanied by post-operative bleeding lasting up-

wards of one month. Following the first revision blood transfusion therapy was attempted but no beneficial results were noted. Other than a liability to easy bruising, neither hemarthroses nor any other hemorrhagic manifestations were reported.

The past history revealed nothing of significance, but the patient stated that there were both male and female bleeders in his family, as shown in Figure 1.

Physical examination was unrevealing save for the well-healed amputation stump. Routine laboratory studies were all within normal limits.

METHODS

Blood was collected as follows: Two syringes were used. A small amount of blood was aspirated into the first syringe, and this was discarded. With the needle left in the vein the second syringe was then applied, and the necessary blood withdrawn. Uncoated glassware was used throughout the study except as otherwise indicated. For the preparation of plasma, one part of 0.1 molar potassium oxalate was used to nine parts of blood. All clotting procedures were carried out at 37° C.

Coagulation times of whole blood were performed by the Lee-White three-tube method, but the serum prothrombin determinations were obtained from a fourth tube which had not been tipped or otherwise manipulated. Plasma prothrombin was estimated according to the techniques of Ware and Stragnell (9), and by the Quick method (10). Serum prothrombin was determined four hours after clotting, by the method of Ware and Stragnell (9). Fibrinogen and labile factor were measured by the method of Quick (10), proconvertin according to Owren and Aas (11), and anti-thrombin as described by Kay, Hutton, Weiss, and Ochsner (12). Excessive antithromboplastic activity was excluded as accomplished by Lewis and Ferguson (13) using Difco® thromboplastin in serial dilution. The thrombin generation test was performed according to the method of Pitney and Dacie (14). In this test plasma is recalcified, and at measured intervals aliquots of the mixture are added to tubes containing fibrinogen solution. The clotting time of each fibrinogen sample thus treated indicates the rate of thrombin generation in the recalcified plasma.

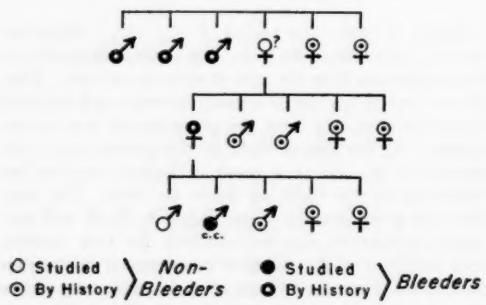

FIG. 1. FAMILY TREE OF PATIENT C. C.

TABLE I

Patient's blood coagulation profile

		Normal values
Platelets	230,000/cu. mm.	200,000–400,000
Coagulation time	22–32 minutes	8–20 minutes
Plasma Prothrombin (Quick)	90%	80–110%
Plasma Prothrombin (Ware and Stragnell)	80–100%	75–110%
Serum Prothrombin	45–71%	5–35%
Labile factor	100%	70–100%
Proconvertin	100%	70–120%
Fibrinogen	625 mg. %	250–400 mg. %
Clot retraction	normal	
Rumpel-Leede	negative	

Thromboplastin generation was studied by the technique of Biggs and Douglas (15). The basis of this test is the finding that in the presence of adequate calcium ion, a mixture of normal platelets, serum, and deprothrombinized plasma produces a potent thromboplastin. This thromboplastin can be measured by its activity in shortening the clotting time of normal recalcified plasma.

Platelet-poor native plasma was prepared from the patient's and normal blood in the following manner: All glassware was silicone-coated, and needles were treated with Arquad®. Processing was accomplished at 0° C. The blood was first centrifuged at 2,500 rpm for ten minutes, and the plasma was pipetted into a second set of tubes. These were then spun at 30,000 rpm in an International Refrigerated Centrifuge for thirty minutes. The resulting plasma, free of anticoagulants, was used promptly for tests of platelet function.

For the preparation of platelet suspensions, the blood was collected into silicone-coated glassware. Sequestrene® was used as the anticoagulant, 0.4 cc. of a 10 per cent solution having been used per 15 cc. of blood. The blood was first centrifuged at 1,000 rpm for ten minutes, and the platelet-rich plasma separated. The platelets were collected by centrifugation at 3,000 rpm for thirty minutes, and washed twice in isotonic saline. They were finally suspended in a volume of saline such that a concentration of about 100,000 per cu. mm. resulted.

Platelet activity was determined by adding 0.9 cc. of native plasma to 0.1 cc. of platelet suspension. Four hours after clotting of this mixture, the prothrombin content of the resulting serum was measured as described above.

Both for measurement of activity of various blood derivatives in correcting the patient's clotting defect, and for the determination of AHF, PTC, and PTA in the patient's blood, a similar technique was used. In each case, the appropriate plasma, serum, or protein fraction was diluted as indicated in *Results*, and 0.1 cc. was added to 1.9 cc. of the freshly drawn whole blood being used as the indicator of activity. The mixture was allowed to clot, and serum prothrombin measured after four hours of incubation. Studies on the clotting times of such mixtures were not undertaken because it was felt that the inaccuracy of the clotting time was too great to permit adequate interpretation.

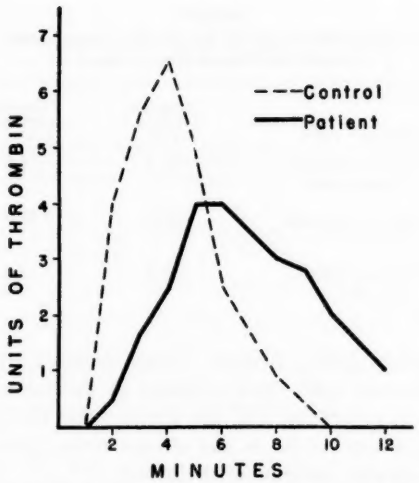

FIG. 2. EVOLUTION OF THROMBIN IN PATIENT'S RECALCIFIED PLASMA COMPARED TO NORMAL CONTROL

The concentrated PTC preparation used in these studies was prepared by adsorption of Cohn's fraction IV with barium sulfate, and elution of this barium sulfate with sodium citrate (4).

The ability of the patient's plasma and serum to activate purified prothrombin, was kindly measured by Johnson, according to her recently published technique (16).

Ammonium sulfate fractionation of normal plasma was accomplished as outlined by White, Aggeler, and Glendening (4). The resulting plasma fractions and plasma fractions of Cohn [1] were tested in concentrations proportional to their original plasma values.

RESULTS

A. *Identification of a new coagulation disorder.*

Coagulation of the patient's blood. Table I shows the results of clotting studies performed on the patient's blood. Of these tests, only the clotting time and the prothrombin consumption were abnormal. However, neither of these abnormalities was profound. A further reflection of the impaired blood coagulation was seen in the evolution of thrombin in recalcified plasma; the appearance of thrombin was delayed, and the peak concentration attained was somewhat below normal (Figure 2).

The clotting defect is more nicely demonstrated in the thromboplastin generation test, in which it is evident that there is marked retardation of thromboplastin formation (Figure 3). Characteristic patterns were obtained by substituting singly, reagents prepared from the patient's blood. Abnormal curves were obtained when either the patient's deprothrombinized plasma or serum was used; but when his platelets alone were substituted, thromboplastin generation was normal. These findings differ from those of both hemophilia and PTC deficiency. In hemophilia thromboplastin generation is abnormal only when deprothrombinized plasma is substituted; in PTC deficiency the test is positive only when serum is substituted. In PTA deficiency any substitution produces improvement (17). The results of this test also indicate that platelet function was normal, and this finding is confirmed by other studies of platelet activity.

The activation of purified prothrombin by the patient's serum and plasma is shown in Figure 4. There is considerably reduced activity in the plasma, but the serum gives essentially a similar curve. This pattern is similar to that found in hemophilia, but differs from the one obtained in PTC deficiency where the serum is even less active than plasma.

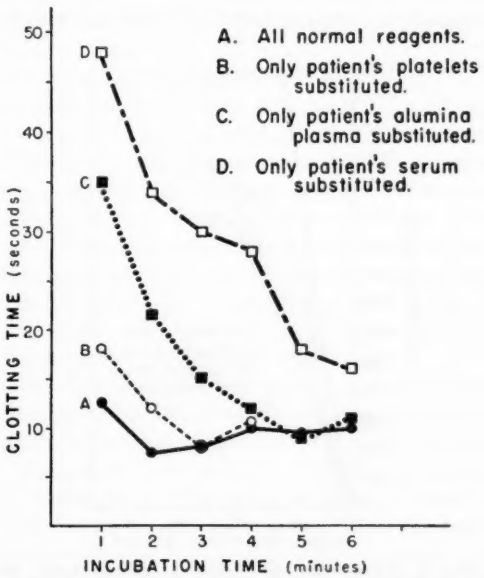
A. All normal reagents.
B. Only patient's platelets substituted.
C. Only patient's alumina plasma substituted.
D. Only patient's serum substituted.
CLOTTING TIME (seconds)
INCUBATION TIME (minutes)

FIG. 3. ACTIVITY OF PATIENT'S REAGENTS WHEN SUBSTITUTED SINGLY IN THE THROMBOPLASTIN GENERATION TEST

[1] Kindly provided by the Cutter Laboratories, Berkeley, California.

TABLE II

Studies of platelet function *

Mixture	Serum prothrombin (%)
Normal plasma + saline	100
Normal plasma + normal platelets	24
Normal plasma + patient's platelets	12
Patient's plasma + saline	100
Patient's plasma + normal platelets	55

* Residual serum prothrombin 4 hours after clotting, when 0.1 cc. of platelet suspension was added to 0.9 cc. of "native" plasma.

A test for antithromboplastin was negative (Table III). In each case the addition of the diluted thromboplastin was as effective against the patient's plasma as in the control. The antithrombin titer of both the patient's and control plasma was 1:64.

Platelet studies. As can be seen from Table I, platelets were normal in number, and clot retraction was adequate. That the platelets likewise had normal clotting activity is shown in Table II. The patient's platelets restored normal prothrombin consumption to normal, platelet-poor native plasma. In contrast, normal platelets added to the patient's platelet-poor native plasma did not result in comparable prothrombin consumption, although an excess of normal platelets was able to improve clotting further. These findings are in conformity with those obtained in the thromboplastin generation test, and indicate that the clotting defect resides in the plasma rather than in the cellular elements of the blood.

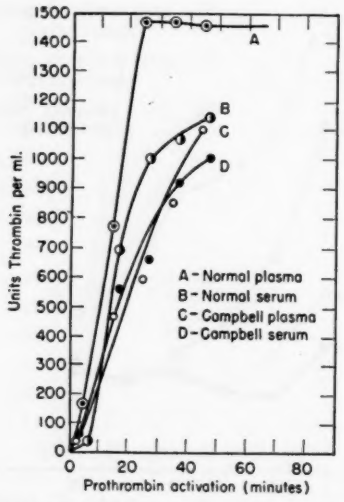

FIG. 4. ACTIVATION OF PURIFIED PROTHROMBIN BY 1:20 DILUTIONS OF PATIENT'S PLASMA AND SERUM IN THE PRESENCE OF PLATELETS, COMPARED TO NORMAL CONTROL (COURTESY OF JOHNSON, S. A. AND SEEGERS, W. H.)

TABLE III

Clotting time in seconds, of patient's plasma when treated with diluted thromboplastin

Reagent added	Patient's clotting time	Control clotting time
Thromboplastin (undiluted)	14.0	14.5
Thromboplastin (1:100)	35.0	37.0
Thromboplastin (1:200)	50.0	47.0

Studies of previously described plasma thromboplastic factors. The activity of the patient's plasma in improving clotting of hemophilic, PTC-deficient, and PTA-deficient blood is shown in Table IV. In each case, the ability of the patient's plasma to improve prothrombin consumption compares favorably to that of normal plasma. Similarly, hemophilic, PTC-deficient, and PTA-deficient plasma and serum were effective in restoring normal prothrombin consumption to the patient's blood, as shown in Table V.

B. *Properties of the deficient factor.*

Stability. The stability of the clotting factor lacking in the present patient is shown in Table

TABLE IV

Activity of various plasmas and sera against patient's clotting disorder *

Preparation added	Improvement in prothrombin consumption (% control–% test)
Normal plasma	22–38
Normal serum	52
Hemophilic plasma	26
PTC-deficient plasma	28
PTC-deficient serum	35
PTA-deficient plasma	27
Patient's plasma stored frozen	0
Patient's serum	9
Saline	0

* One-tenth cc. of undiluted plasma or serum was added to 1.9 cc. of the patient's blood, and the serum prothrombin determined 4 hours after clotting. The control is the serum prothrombin of the patient's unmodified blood incubated at the same time.

TABLE V

Activity of patient's plasma against thromboplastic disorders *

Combination of:	Serum prothrombin (%)
Hemophilic blood alone	100
2% patient's plasma + hemophilic blood	34
2% normal plasma + hemophilic blood	42
PTC-deficient blood alone	80
5% patient's plasma + PTC-deficient blood	24
5% normal plasma + PTA-deficient blood	22
Patient's plasma + PTA-deficient blood	Same as normal†

* In each case 0.1 cc. of the patient's plasma appropriately diluted was added to 1.9 cc. of test blood, and the serum prothrombin determined 4 hours after clotting.
† *Rosenthal, R. L.:* Personal communication.

VI. In each case, 0.1 cc. of undiluted plasma was added to 2 cc. of the patient's whole blood, and the serum prothrombin determined four hours after clotting. The activity of normal plasma is stable with storage for at least one week at room or refrigerator temperature; indeed, the activity of the plasma appears to be somewhat enhanced after storage. However, at 56° C. it is rapidly destroyed. Thus, in its storage properties the new factor resembles PTC, although it is even less stable to heat. In contrast, AHF is extremely storage-labile, and relatively heat-stable. Comparable data are not available for the stability of PTA.

Fractionation behaviour. The activity of various preparations of nomal plasma is indicated in Table VII. The activity is not removed by repeated treatment of plasma with barium sulfate. Passage through a Seitz filter containing 50 per cent asbestos removes a considerable amount of activity, but those with lower concentrations have little effect. In these respects its behaviour is similar to that of AHF.

TABLE VI

Stability of plasma activity *

Type of plasma	Improvement in prothrombin consumption (% control–% test)
Stored at 4° C. for 1 week	37
Stored at 20° C. for 1 week	34
Heated at 56° C. for 5 minutes	2

* One-tenth cc. of plasma was added to 1.9 cc. of the patient's blood, and the serum prothrombin determined 4 hours after clotting. The control is the serum prothrombin of the patient's unmodified blood incubated at the same time.

TABLE VII

Activity of plasma fractions *

Plasma fraction added	Improvement in prothrombin consumption (% control–% test)
Barium sulfate plasma	28
Fraction I	0
Fraction II	0
Fraction III	21
Fraction IV	30
Fraction V	0
Seitz-filtered plasma (50% asbestos)	19
PTC concentrate (8-fold concentrate)	42
PTC concentrate (reconstituted)	10
Precipitate from 25% ammonium sulfate plasma	8
Precipitate from 33% ammonium sulfate plasma	23
Precipitate from 50% ammonium sulfate plasma	30
Supernatant from 50% ammonium sulfate plasma	10

* Each fraction was used in its normal plasma concentration. One-tenth cc. of the fraction was added to 0.9 cc. of the patient's blood and the serum prothrombin determined 4 hours after clotting. The control is the serum prothrombin of the patient's unmodified blood incubated at the same time.

Although the new factor differs from PTC with respect to its adsorption on barium sulfate, there is similarity in the Cohn fractions wherein they are found. Of the various fractions tested, only fractions III and IV were active. AHF, in contrast, is found in fraction I, and PTA in III and IV (17). Likewise, the new factor resembles PTC in that 50 per cent ammonium sulfate saturation is required for maximal precipitation from normal plasma. It differs from AHF and PTA which are found in the 33 per cent ammonium sulfate fraction (18). An unexpected finding was that of activity in a concentrated PTC preparation which was prepared from an eluate of barium sulfate used to adsorb fraction IV.

Survival of the factor in vivo. It was anticipated that the corrective effect of normal plasma on the patient's blood clotting *in vivo* would be comparable to its *in vitro* activity. This has not proved to be the case. The following forms of transfusion therapy were tried: 250 and 500 cc. of fresh, citrated plasma, 100 cc. of heparinized serum, 350 cc. of whole blood by direct transfusion, and 100 cc. of 10 per cent fraction IV. In each case there was little or no change in either the patient's whole

blood clotting time or prothrombin consumption. The minimal changes that were noted had all disappeared within an hour after completion of the transfusion.

DISCUSSION

The above data indicate that the following conclusions are justified: *1*) The coagulation defect in our patient appears to result from defective plasma thromboplastin formation. This is reflected in the diminished utilization of prothrombin in the course of his blood clotting, and the retarded generation of thromboplastin in the Biggs and Douglas test. *2*) The origin of the defect does not reside in the platelets; for the patient's platelets behave normally both in the thromboplastin generation test and in their ability to restore normal prothrombin consumption to "native" normal plasma. Moreover, in both of these situations, normal platelets failed to correct the patient's clotting defect. *3*) The disorder does not result from lack of antihemophilic factor, as shown by several tests. Mixing the patient's blood with hemophiliac blood produces mutual correction of clotting in each case. In the thromboplastin generation test substitution of either the patient's serum or deprothrombinized plasma resulted in an abnormal curve, whereas in the hemophiliac it is only the plasma substitution which gives an abnormal result. The corrective activity of normal plasma for our patient was stable on storage, but labile to heat. AHF is storage-labile and relatively heat-stable. For our patient corrective activity was found in serum, Cohn's fractions III and IV only, and in the precipitate from 50 per cent ammonium sulfate saturated normal plasma. AHF is not found in serum, is mainly concentrated in Cohn's fraction I, and requires only 33 per cent ammonium sulfate for its precipitation from normal plasma. *4*) The differentiation from PTC deficiency is likewise sharp. Here, too, there is mutual correction of the clotting defect with mixing of respective bloods. PTC deficiency also shows a characteristic and different pattern in the thromboplastin generation test in that it produces an abnormal curve only when serum is substituted. In the activation of purified prothrombin the present patient's plasma and serum were both equally defective; whereas in PTC deficiency the serum is even more defective than plasma. The activity of normal plasma against our patient's defect is not removed by adsorption with barium sulfate, or by low asbestos Seitz filtration; whereas PTC is completely removed by either of these procedures.

Differentiation of the present disorder from PTA deficiency is primarily based on the tests of mutual correction of one blood with plasma of the other. PTA activity is maximally precipitated with 33 per cent ammonium sulfate, and further differentiation is provided by the thromboplastin generation test in which substitution of either normal plasma or serum provides correction for PTA deficiency.

Clinically the thromboplastic disorders differ in their response to transfusion. In hemophilia the effect of blood or plasma is quite transient, usually lasting less than 24 hours. In PTC deficiency there is a considerably more prolonged effect, and coagulation tests may not return to control levels for three weeks following a transfusion (4). An intermediate response is seen in PTA deficiency (17) in that transfusion produces an effect lasting about one week. Our patient was quite refractory to any form of transfusion therapy.

On the basis of these findings, it would appear likely that there is an additional plasma thromboplastic component which is distinct from AHF, PTC, and PTA. However, the introduction of a new coagulation factor must be made with greatest caution, and artefact must be carefully excluded. In the present case, three such possibilities warrant serious consideration. *1*) Exclusion of a deficiency in one of the previously known factors is based on the traditional method of measuring the ability of the patient's plasma or serum to correct clotting in known defective bloods. The underlying assumption in such a method is that there can be correction only by supplying the substance specifically lacking. Such an assumption does not admit the possibility of nonspecific correction, a simple example of which would be the corrective activity of trypsin on hemophilic blood (18). It has been our experience that prothrombin consumption in mixtures of normal bloods may exceed that obtained in either alone. As the clotting in our patient was only moderately abnormal, the activity of the other abnormal plasmas in correcting his defect could possibly have resulted from non-specific activity. On the other

hand, it is less likely that his plasma could improve so markedly the severely abnormal bloods of the patients with known disorders, on a non-specific basis. At least in the cases of established hemophilia and PTC deficiency, such non-specific correction has not been found. *2*) A combined but partial deficiency of two of the previously described factors could result in a situation by which addition of a blood defective in one of them would result in improved clotting. Such a case has actually been reported by Soulier and Larrieu (19), in which hemophilia was combined with PTC deficiency. However, each deficiency was clearly demonstrated by a suitable assay procedure. It would seem unlikely that a major defect of a factor would be missed by the present techniques, as the patient's plasma was used only in high dilution when tested against the other defective bloods. *3*) The present syndrome could be produced by a circulating anticoagulant. The failure of transfusion to improve the patient's clotting underlines this possibility, although the active agent could, like prothrombin, be very rapidly metabolized (*20*). Such an hypothetical anticoagulant would have to be present in extremely low concentration, for as little as 1 per cent normal plasma produced appreciable improvement of the patient's prothrombin consumption *in vitro*. All attempts to demonstrate such an anticoagulant were unsuccessful, nor is it easy to see where in the coagulation process it could act without at least partially neutralizing one of the other known thromboplastic components. In some of the reported patients with circulating anticoagulants, it has been demonstrated that there is specific neutralization of AHF (15, 21). Nevertheless, these considerations indicate the need for independent confirmation before the suggested new coagulation factor can be accepted without reservation. This has been provided to a certain degree by the finding of an additional patient whose blood resembles that of ours. Rosenthal (17) has studied a blood evidently defective in thromboplastin formation which is corrected by plasma of patients with hemophilia, PTC deficiency and PTA deficiency, but not by the plasma of our patient.

On the assumption that a new coagulation factor has indeed been unearthed, the question of terminology is immediately presented. Several techniques of nomenclature are in current use. In one, the new disorder is given the name of the first family in which it was detected (Moena Anomaly, Christmas Disease). A second method attempts to apply descriptive terms to the missing factor as it appears to function in the clotting mechanism, thus: plasma thromboplastin component (PTC), plasma thromboplastin antecedent (PTA). Such terms imply that the place of the factors in the coagulation scheme is established, although the accompanying data are less conclusive. Moreover, this system does not have the flexibility to incorporate newly discovered factors. The system of nomenclature proposed by Soulier and Larrieu (*7*) is perhaps more satisfactory for the present needs. According to this scheme the various thromboplastic disorders are lettered, so that classical hemophilia is "hemophilia A," PTC deficiency is "hemophilia B," PTA deficiency is "hemophilia C," and the present disorder "hemophilia D." The difficulty involved in Soulier and Larrieu's system is the implication that all these conditions are variations of the same disease, whereas the data indicate that they are distinctly different disorders with similar clinical pictures. Therefore, Aggeler, White, and Spaet (*22*) prefer to retain the name "hemophilia" for the classical disease and append Greek numerical prefixes for the other thromboplastin deficiency diseases. PTC deficiency thus becomes deuterohemophilia, PTA deficiency tritohemophilia and the present disorder tetartohemophilia. These authors suggest a uniform nomenclature for the corresponding coagulation factors in which the generic term for the group is "plasma thromboplastic factor (PTF)." Each factor would be identified by an appended letter as follows: PTF-A (AHF), PTF-B (PTC), PTF-C (PTA), and PTF-D (present factor).

SUMMARY

A patient is reported with a blood clotting abnormality consisting of a prolonged coagulation time, reduced prothrombin utilization, and retarded generation of plasma thromboplastin. No anticoagulant was demonstrable, and all previously described coagulation factors were normally present. Normal plasma was corrective for the defect, and activity was found in serum, Cohn's fractions III and IV, and in the 50 per cent ammonium sulfate saturation precipitate of normal plasma. The

activity was heat-labile, storage-stable, not adsorbed on barium sulfate, and removed only by high concentration asbestos Seitz filters. Transfusion of blood or its fractions did not improve the patient's clotting.

It is tentatively suggested that there is a previously undescribed coagulation factor concerned with the formation of plasma thromboplastin. It is proposed that this new factor be called, "plasma thromboplastic factor D (PTF-D)," and that its deficiency be named, "tetartohemophilia."

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THE ROLE OF EXOGENOUS LIPIDS IN THE HYPERLIPEMIA AND HYPERCHOLESTEREMIA OF NEPHROTIC RATS[1]

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The renal disease, ascites, subcutaneous edema, and hyperlipemia induced in rats by injection of rabbit anti-rat kidney serum comprise a syndrome which closely resembles the nephrotic state as it occurs in humans (1). This provides a unique opportunity to study the mechanism(s) of the disturbed lipid metabolism of the nephrotic state. In previous studies from this laboratory it was found that the hypercholesteremia of nephrotic rats cannot be ascribed either to an increased intestinal absorption of dietary cholesterol (2) or to an increased hepatic synthesis of endogenous cholesterol (3). In the present study the role of exogenous lipids is further assessed, the data showing that the development of experimental nephrotic hyperlipemia and hypercholesteremia is independent of dietary fat.

METHODS AND MATERIAL

Twenty male rats (Long-Evans strain), were injected intravenously with 0.5 ml. of rabbit anti-rat kidney serum (1) on each of two successive days. Seven of the rats (Group I) were fed a fat and sterol-free ration for five days prior to injection of immune serum, and continued to ingest this ration. Eight rats were fed stock laboratory ration (Group II) and five others (Group III) ingested stock ration supplemented with cholesterol (2 per cent of the diet) and cholic acid (1 per cent of the diet). The cholesterol and cholic acid-supplemented diet also was fed to six normal rats (Group IV) which were not injected with anti-kidney serum. A fifth group of ten other control rats (Group V) were fed stock ration.

Each rat was bled from the tail six days after the injection of immune serum, and assay made according to previously described methods of the plasma concentrations of total cholesterol (4) and total lipids (5). Four days later similar determinations again were made in the rats ingesting stock ration (I) and the fat-sterol-free diet (II).

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RESULTS

Six days after the administration of immune serum all of the injected rats exhibited ascites, and lipemic-turbidity of their plasma. Table I presents the averages and ranges of plasma total lipids and total cholesterol values obtained from the nephrotic rats fed stock ration (Group II), those fed the diet free of fats and sterols (Group I), and the normal rats fed stock ration (Group V). The data show that the rats fed the fat-sterol-free diet developed a degree of hyperlipemia and hypercholesteremia equal to, or slightly greater than that developed by the nephrotic animals ingesting stock ration. The nephrotic rats of both groups exhibited a similar degree of ascites, subcutaneous edema, and grossly apparent turbidity of the plasma samples.

The ingestion of a ration containing excess cholesterol and cholic acid has been shown to induce chronic hypercholesteremia in rats (6, 7), due to the increased intestinal absorption of cholesterol effected by simultaneous administration of cholate (8). The effect of ingesting this supplemented diet for six days upon the hyperlipemia and hypercholesteremia of nephrotic rats and of the plasma lipids of normal rats is presented in Table II. It can be seen that both the hyperlipemia and hypercholesteremia were of much greater magnitude in the nephrotic rats (Group III) fed the cholesterol and cholate supplement, than in the nephrotic animals fed stock ration without the supplement (Group II). The normal control rats which ingested the cholesterol and cholate supplement (Group IV) also developed a moderate rise of plasma total lipids and total cholesterol in this interval. However, the magnitude of the hypercholesteremia was of lesser degree than the difference observed in the nephrotic rats which ingested the supplemented diet (Group III) when the latter are compared with the nephrotic rats which were fed the stock ration

TABLE I

Effect of sterol free diet upon development of hyperlipemia and hypercholesteremia of nephrotic rats *

Group	Type of rat	No. of rats	Diet	Average weight (*Gm.*)	Average plasma concentration (*mg./100 cc.*) Day 6 Cholesterol	Day 6 Total lipids	Day 10 Cholesterol	Day 10 Total lipids
I	Nephrotic	7	Fat and Sterol free	120	196 (109–266)	968 (291–1710)	485 (425–530)	2073 (1650–2700)
II	Nephrotic	8	Stock Ration	125	157 (108–324)	832 (327–1710)	409 (299–518)	1298 (820–1670)
V	Normal	10	Stock Ration	140	48 (39–63)	183 (135–227)		

* Range of values in parentheses.

(Group II). The ingestion of the cholesterol-cholate supplement did not otherwise appear grossly to affect the nephrotic state of the rats of Group III.

DISCUSSION

The present finding that the hypercholesteremia of the nephrotic rat occurs in the complete absence of dietary lipid is in accord with our earlier observation (2) that there is no increase in the rate of intestinal absorption of cholesterol in this disorder. In view of the fact that there also does not appear to be any increase in the rate of endogenous manufacture of cholesterol by the liver (3), it appears that the hypercholesteremia can only be due to a failure primarily of the liver to remove plasma cholesterol at its usual rate (9). In confirmation of this latter view is the present observation that additional intestinal absorption of cholesterol (8) by cholesterol-cholate feeding leads to a greater retention of cholesterol in plasma of nephrotic rats than in that of normal rats. The disorder then appears to be one in which cholesterol is "trapped" in plasma. Whether the excess cholate in plasma also observed in this sydrome (9, 10) causes the hypercholesteremia, as it was found to do in biliary obstruction (11), or whether it simply intensifies the degree of hypercholesteremia cannot be stated with certainty at this time. The tendency of the remainder of the lipid component of plasma to behave exactly as cholesterol does in all of these studies of course suggests that the mechanism responsible for the plasma retention of the latter is also operative in the retention of the former.

SUMMARY

The development of hyperlipemia and hypercholesteremia was studied in nephrotic rats fed a normal diet, a ration free of fats and sterols, and

TABLE II

Effect of excess dietary cholesterol upon hyperlipemia and hypercholesteremia of nephrotic rats

Group	Type of rat	No. of rats	Diet	Average weight (*Gm.*)	Average plasma concentration (*mg./100 cc.*)† Cholesterol	Total lipids
II	Nephrotic	8	Stock Ration	125	157 (108–329)	832 (327–1710)
III	Nephrotic	5	Stock and Sterols*	150	446 (328–653)	1307 (855–2180)
IV	Normal	6	Stock and Sterols*	135	138 (68–255)	645 (400–800)
V	Normal	10	Stock Ration	140	48 (39–63)	183 (135–227)

* 2 per cent cholesterol and 1 per cent cholic acid.
† Figures in parentheses refer to range of values.

a ration supplemented with cholesterol and cholic acid. The results indicate that the development and maintenance of hyperlipemia and hypercholesteremia in the nephrotic rat are of endogenous origin and that increased retention of lipids occurs in the plasma of such rats. It is suggested that the hyperlipemia and hypercholesteremia of nephrotic rats are due to a diminished rate of elimination of lipids from their plasma.

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THE FATE OF HYDROCORTISONE-4-C^{14} IN MAN [1]

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This study deals with the dynamic behavior of hydrocortisone in man and the relationship of the metabolic fate of this principal adrenal hormone to the physiologic or pathologic state of the experimental subject. The period during which the hormone persists in the tissues, the rate of its excretion, and the relationship between the two have been measured by precise and discriminating methods. Furthermore, the details of the metabolic transformations and the rate at which these occur have been discussed and correlated with some known biologic effects of the hormone. These studies have been possible as a result of the availability of ring A labeled hydrocortisone through a cooperative effort sponsored by the Endocrinology Study Section of the U. S. Public Health Service (1).

Previous work in our laboratories employing radioactive cortisone labeled with tritium yielded information concerning the metabolites of cortisone and the routes by which they are eliminated. Exact quantitative evaluation of the total metabolites in terms of the amount given was difficult because of errors inherent in the methods employed for the analysis of tritium in very crude extracts. Other studies with C^{14} labeled testosterone, estrone, estradiol, progesterone, and desoxycorticosterone have been reported, and in general, these experiments showed a rapid elimination of the hormonal metabolites as conjugated substances in the urine (2). These analyses were possible because of the greater facility and accuracy of carbon-14 measurement. From the experience gained in these studies, it was possible to refine the experimental design so as to supply information concerning the following aspects of hydrocortisone metabolism.

First, the amount, the rate, and the route of excretion were established. Second, the problem of "tissue demand" as evidenced by the alteration in these excretory processes was explored in the complete absence of endogenously produced hormone. Third, these findings were compared with those obtained when the tissues were saturated with hormone. Fourth, the constancy of these processes was studied in different subjects with alterations in their physiological state as a result of disease. Fifth, the influence of the amount of hormone administered on these aspects of metabolism was defined. Sixth, the question of hormone breakdown ("utilization"), as indicated by the loss of C^{14} from ring A, was measured by the most delicate method available.

EXPERIMENTAL

The subjects in this study were patients on the metabolic ward of the James Ewing Hospital Unit of Memorial Center. Pertinent clinical information is summarized in Table I. With one exception, urine collections were made through a catheter to insure accuracy during the early portion of each study. Complete collections of urine and feces were made for a period of five days after the administration of the labeled material. Thirty to fifty ml. samples of blood were taken into tubes containing heparin at several time intervals for the determination of the quantity of hormone and its metabolites in circulation. In one patient, a sample of spinal fluid was obtained at 8 hours.

Hydrocortisone-4-C^{14} was supplied as a crystalline material with a specific activity of 4.69 μc per mg. The purity of the product had been established by the conventional methods of column chromatography, sulfuric acid chromogens and paper chromatography together with radioautography. In all experiments, the hormone

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TABLE I

Description of subjects

Subject	Age	Sex	Diagnosis and therapy	Exp.	Hydrocortisone administered	Remarks
F1	63	F	Chronic lymphatic leukemia 7 yrs. Radiation 6 yrs. and 6 mos.	1	0.855 μc (0.25 mg.)	Methadone addition Liver function normal
F2	43	M	Multiple benign lipomata 4 yrs.	2	0.94 μc (0.25 mg.)	Good general condition Liver function normal
				3	0.954 μc (100 mg.)	
F3	69	F	Breast cancer 2 yrs. Radical mastectomy 2 yrs. Recurrent disease 6 mos. Radiation 4 mos.	4	0.948 μc (100 mg.)	Good general condition Liver function normal
F4	48	F	Breast cancer 3 yrs. Radical mastectomy 3 yrs. Recurrent disease 2 yrs. Radiation 2 yrs. Castration 9 mos. Adrenalectomy 6 mos.	5	0.902 μc (0.25 mg.)	All hormone therapy stopped 48 hrs. before study. Usually maintained on oral cortisone
				6	0.790 μc (0.25 mg.)	Study performed while subject was receiving 100 mg. hydrocortisone i.v. each day

was administered intravenously. A sterile solution for the study was prepared immediately prior to infusion by the following procedure. The steroid was transferred into sterile ampules so that each ampule contained approximately 1 μc. The contents of the ampule were dissolved in 2 ml. of absolute ethanol which was added with proper precautions to approximately 150 ml. of sterile isotonic sodium chloride solution. An additional 2 ml. of absolute ethanol, used to rinse the ampule, was added to the infusion solution. When non-radioactive carrier hormone was used, 100 mg. of hydrocortisone, especially purified for this purpose, was transferred in alcohol solution to the infusion vessel. A small portion of the infusion solution was removed for analysis of the radioactivity. The quantity of solution administered to the patient was determined by weighing the infusion bottle before and at the termination of the infusion. In every instance, the solution of the radioactive hormone either with or without carrier was introduced over a period of exactly 30 minutes.

All radioactivity measurements were carried out independently by two different procedures. In the one, (Method A) the material was plated on planchets of 4.9 cm^2. area containing lens paper. These were then counted in a windowless flow gas counter and the data obtained were corrected to infinite thickness. A portion of the infusion solution was diluted with saline and plated as described. The radioactivity of this solution was referred to a National Bureau of Standards C^{14} reference standard ($Na_2C^{14}O_3$)—plated and counted in the same manner. This provided an accurate determination of the amount given to each patient. In order to permit a direct comparison of the infusion solution with the urine samples, an "infusion standard" with the plating characteristics of urine was prepared by dilution of a portion of the original hydrocortisone-4-C^{14} infusion solution with urine. The urine samples, themselves, were directly plated without dilution, extraction or further manipulation. In the other method of radiocarbon measurement (Method B), the infusion solution, urine, or extracts in organic solvents were made to a known volume, small portions were accurately removed and transferred to planchets of 5, 10 or 18 cm^2. area. The amount spread on the planchet was in the infinitely thin range; radioactivity was measured in a windowless flow gas counter. Both methods of radiocarbon measurement were intercalibrated by means of the National Bureau of Standards C^{14} reference standard.

In Experiment 1, samples of expired air were collected by exhalation through a 0.5 N sodium hydroxide solution. Several collections obtained for 30-minute intervals at various times during the first two days of the experiment were combined; a control sample was collected in the same fashion prior to the administration of any radioactivity. The control and experimental carbon dioxide collections were then separately converted to elemental carbon for measurement of radiocarbon by the method of Anderson and Libby (3). This procedure is capable of detecting 10^{-4} per cent of the dose used in these experiments.

Blood and spinal fluid samples were delivered into 1:1 acetone-alcohol, the precipitate was thoroughly extracted with the organic solvent and these extracts were counted, in many instances by both procedures. For the measurement of unchanged, unconjugated hydrocortisone in blood by the method of isotopic dilution, the following procedure was used in Experiment 2 (Subject F2). A 63 ml. sample of blood was withdrawn 15 minutes after the termination, *i.e.*, 45 minutes after the initiation of the infusion. The blood was extracted as above and to the extract was added 30.2 mg. of highly purified non-radioactive hydrocortisone. A total of 4,000 disinte-

grations per minute (DPM per min.) was present in the extract. The mixture was acetylated with an excess of acetic anhydride in pyridine at room temperature for 48 hours and the hydrocortisone acetate was extracted with ethyl acetate; the solution was washed with dilute alkali, dilute acid and with sodium chloride solution. The solvent was removed and the neutral extract contained 2,100 DPM per min. (54 per cent of the total). The extract was chromatographed on a silica gel-ethanol partition-type column (4) using alcohol and methylene chloride as the mobile phase. Most of the radioactivity was removed from the partition column before hydrocortisone acetate was eluted. The eluates containing hydrocortisone acetate were combined and recrystallized from acetone; the product melted 206 to 210°; specific activity = 15 DPM per min. per mg. After a second recrystallization, the m.p. was 214 to 217° and the specific activity was unchanged. The amount of material remaining was insufficient for further recrystallization.

The extraction of "free" steroids from urine was made by a minor modification of the procedure of deCourcy, Bush, Gray, and Lunnon (5). The urine was extracted four times, each with half its volume of chloroform, and twice with similar quantities of ether. The combined chloroform extracts were then washed three times with ⅛ volume of 5 per cent sodium hydroxide solution containing 5 per cent sodium chloride, followed by three washes with ⅛ volume of 25 per cent sodium chloride solution. The alkaline and saline extracts were backwashed with the two ether extracts. The combined chloroform and ether solutions were dried over sodium sulfate, the solvent was removed and the residue was diluted to volume with ethanol.

RESULTS

Six individual studies of the metabolism of hydrocortisone-4-C^{14} were made in four subjects. Two subjects, one a man (F2) and the other a woman, (F1) each received a tracer quantity of hormone, approximately 0.25 mg.; on another occasion, the same amount of labeled hydrocortisone together with 100 mg. of carrier hormone was given to the same male subject, F2. This experiment permitted an evaluation of the effect of the quantity of hormone given on its subsequent fate in the same individual. A female subject (F3) also received 1 μc of hydrocortisone-4-C^{14} together with 100 mg. of carrier hormone. Two studies were carried out on the fourth subject (F4), a

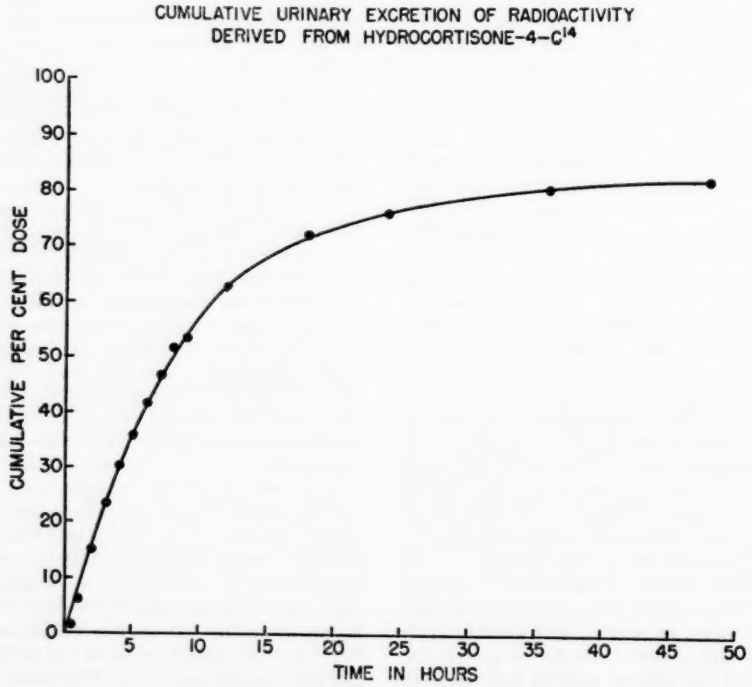

FIG. 1. THE CUMULATIVE URINARY EXCRETION OF RADIOACTIVITY IN EXPERIMENT 4 (SUBJECT F3) AFTER THE INTRAVENOUS ADMINISTRATION OF 100 MG. (0.948 μC) OF HYDROCORTISONE-4-C^{14} DURING 30 MINUTES

woman who was both oophorectomized and adrenalectomized. In the first of these, all hormone therapy was withdrawn for 48 hours before, and 24 hours after, the intravenous administration of a tracer amount of hydrocortisone. This procedure permitted an evaluation of the metabolism of the hormone when the tissues of the recipient were depleted of this substance. A second study on this same subject was carried out while she was receiving 100 mg. of hydrocortisone daily by continuous intravenous infusion. In this instance, the non-radioactive hydrocortisone infusion was interrupted for ½ hour while the tracer dose of the radioactive hormone was introduced. The infusion of the unlabeled hormone was then continued for the following 24 hours.

Data on the excretion of hydrocortisone metabolites in the urine are listed in Table II and a typical cumulative excretion curve (subject F3) is shown in Figure 1. In Experiment 1 (F1), 56 per cent was excreted in the urine at the end of 6 hours; 72 per cent was eliminated in one day and 80 per cent over three days. Virtually the same results were obtained in Experiment 2 with a tracer dose administered to the male subject, F2. In Experiment 3, when this same subject was given 100 mg. of carrier hydrocortisone together with radioactive hormone, the large additional quantity of hormone did not appreciably alter the excretory pattern. Fifty-four per cent was excreted in 6 hours, and 84 per cent in one day. The female subject, F3, who was also given 100 mg. of hydrocortisone containing 1 μc of C^{14}, showed an almost identical pattern of excretion in Experiment 4. In Experiment 5, conducted while the adrenalectomized woman, F4, was deprived of all replacement hormone therapy, the excretory pattern was also similar and no significant changes were observed when the same subject received 100 mg. of hydrocortisone daily by continuous intravenous infusion in Experiment 6.

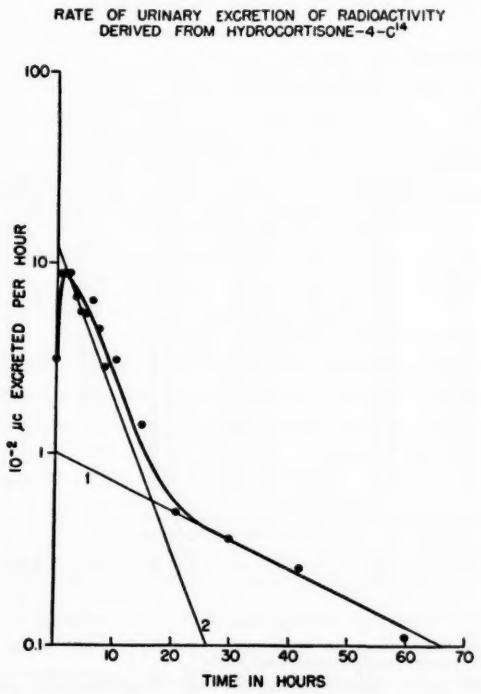

FIG. 2. THE EXCRETION OF RADIOACTIVITY IN EXPERIMENT 4 (SUBJECT F3) AFTER THE INTRAVENOUS ADMINISTRATION OF 100 MG. (0.948 μc) OF HYDROCORTISONE-4-C^{14} DURING 30 MINUTES

The curve has been analyzed for the two principal component rates, 1) with $T/\frac{1}{2} = 22$ hours and 2) with $T/\frac{1}{2} = 4$ hours.

In order to define the rate of excretion of the radioactive products derived from the administered hydrocortisone-4-C^{14}, the radioactivity in the urine was plotted semi-logarithmically against time for a typical experiment (Figure 2). During the first 24 hours after the dose, the data group about a straight line and suggest that the excretion approximates a first-order reaction. The rate of excretion of the products derived from the metabolism of 80 per cent of administered hydrocortisone during the first 24 hours can be characterized by an average half-life (T ½) of 3.6 hours in all subjects studied.

In the four days following hormone administration in subject F3, 9 per cent of the radioactivity was found in an acetone extract of the feces. In view of the high recovery of radioactivity in the urine, it appeared that similar results would be obtained in the other patients; further studies of the fecal metabolites are in progress.

The radioactivity found in the blood at various time intervals in the different subjects is shown in Table III. In a blood sample obtained in Experiment 2, 15 minutes after the end of infusion, a maximum of 13 per cent of the circulating radioactivity was present as unaltered hydrocortisone

TABLE II

Urinary excretion of radioactivity after hydrocortisone-4-C¹⁴

Experiment 1 Subject: F-1 Dose: 0.855 μc (0.25 mg.)				Experiment 2 Subject: F-2 Dose: 0.940 μc (0.25 mg.)				Experiment 3 Subject: F-2 Dose: 0.954 μc (100 mg.)			
Time* *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose	Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose	Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose
0–0.5	0.8	1.0	1.0	0–0.5	1.1	1.2	1.2	0–0.5	3.6	4.6	4.3
0.5–1	3.5	3.8	4.3	0.5–1	4.6	4.6	4.9	0.5–1	5.0	5.6	5.6
1–2	13.7	12.5	15.3	1–2	10.6	11.2	11.1	1–2	10.4	11.8	11.2
2–3	8.6	8.6	10.0	2–3	8.4	9.5	9.5	2–3	9.4	10.5	10.4
3–4	8.6	9.9	10.8	3–4	6.8	8.2	8.0	3–4	9.2	9.2	9.6
4–5	9.1	9.9	11.0	4–5	6.6	7.2	7.4	4–5	6.5	6.2	6.5
5–6	3.8	3.1	4.0	5–6	4.9	6.0	5.8	5–6	6.8	5.0	6.2
6–12	7.2	7.2	8.4	6–12	24.4	21.7	24.6	6–12	12.3	13.2	13.3
12–18	3.2	3.2	3.8	12–18	7.0	7.2	7.6	12–18	10.1	12.5	11.8
18–24	3.2	3.1	3.7	18–24	3.5	2.6	3.3	18–24	4.7	2.8	3.9
24–36	2.1	—	2.5	24–36	3.3	—	3.5	24–36	—	3.8	4.0
36–48	1.7	—	2.0	36–48	1.0	—	1.0	36–48	4.7	—	4.9
Total: 24 hours				Total: 24 hours				Total: 24 hours			
	61.7	62.3	72.3		77.9	79.4	83.4		78.0	81.4	82.8

* Elapsed time in hours following start of infiltration.

TABLE II—*Continued*

Experiment 4 Subject: F-3 Dose: 0.948 μc (100 mg.)				Experiment 5 Subject: F-4 Dose: 0.902 μc (0.25 mg.)				Experiment 6 Subject: F-4 Dose: 0.790 μc (0.25 mg.)			
Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose	Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose	Time *hours*	Method A 10^{-2} μc	Method B 10^{-2} μc	Average % dose
0–0.5	1.6	1.4	1.6	0–0.5	—	—	0.05	0–0.5	1.0	1.1	1.3
0.5–1	4.4	4.3	4.6	0.5–1	7.2	6.6	7.6	0.5–1	5.3	6.0	7.2
1–2	8.8	7.9	8.8	1–2	8.0	7.2	8.4	1–2	10.2	11.2	13.5
2–3	8.8	7.6	8.7	2–3	7.2	5.9	7.2	2–3	8.1	7.2	9.8
3–4	6.7	5.9	6.7	3–4	8.6	7.9	9.2	3–4	4.7	5.1	6.2
4–5	5.6	5.2	5.7	4–6	8.2	7.2	8.5	4–5	5.5	5.9	7.2
5–6	5.4	5.3	5.6	6–7	8.4	7.9	9.0	5–6	5.2	5.1	6.5
6–7	6.4	3.6	5.3	7–8	5.1	5.1	5.6	6–9	5.0	3.9	5.6
7–8	4.5	4.8	4.9	8–12	7.5	7.5	8.3	9–12	4.0	3.5	4.7
8–9	2.8	2.9	3.0	12–18	6.8	4.5	6.3	12–18	3.7	3.4	4.5
9–12	9.5	7.9	9.2	18–24	2.7	2.0	2.6	18–24	3.5	2.7	3.9
12–18	8.5	9.2	9.3	24–36	2.5	1.8	2.4	24–36	1.5	—	1.9
18–24	3.0	4.9	4.1	36–48	1.3	1.4	1.5	36–48	1.0	—	1.3
24–36	4.4	4.1	4.5	48–72	2.0	—	2.2	48–72	0.8	—	1.0
36–48	3.0	0.9	2.1	72–96	0.5	—	0.6	72–96	0.7	—	0.9
48–72	1.3	—	1.4								
Total: 24 hours				Total: 24 hours				Total: 24 hours			
	75.9	70.9	77.5		69.7	61.9	72.6		56.3	55.1	70.5

TABLE III

Radioactivity in blood during and after intravenous hydrocortisone-4-C¹⁴

Exp. 1		2		3		4		5		6	
Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.	Time (*min.*)	C^{14} 10^{-2} μc/L.
15	4.0	15	2.1	15	1.6	30	6.0	30	7.6	30	4.8
30	3.3	30	5.1	30	2.1	60	3.3	75	3.8	75	1.9
120	1.2	45	2.7	50	1.8	105	2.6	130	3.4	130	2.9
		150	1.8	135	1.3						
		180	1.5	250	1.5						

TABLE IV

Conjugated urinary radioactivity and exponential rates of excretion in all subjects

Experiment	Conjugation		Rate of excretion	
	Time (*hours*)	% Conjugated	Component	T 1/2
1	1–2	89	2	2.5
	12–18	99	1	37
2	1–2	96	2	4.5
	6–12	99	1	—
3	1–2	89	2	4
	6–12	98	1	—
4	1–2	81	2	4
	6–7	93	1	22
5	1–2	93	2	3.5
	12–18	96	1	20
6	1–2	81	2	3
	9–12	93	1	20

as demonstrated by the isotopic dilution described. In this short time, most of the hormone had therefore undergone metabolic transformation.

The results obtained by separating the urinary metabolites into "free" and conjugated fractions are shown in Table IV. The data indicate that from 4 to 19 per cent or an average of 12 per cent of the radioactive hormone products excreted during a single hour 30 minutes after completion of the infusion, was present in the "free" or nonconjugated form. After the sixth hour, only 1 to 7 per cent or an average of 3 per cent of the excreted material was still unconjugated.

No radioactivity was detected in a single 10 ml. sample of spinal fluid (subject F 1) obtained 8 hours after the infusion was completed. Analysis of the C^{14} content of the control carbon dioxide sample from subject F1, Experiment 1, gave a net activity of 15.1 ± 0.2 DPM per gram of carbon, a value identical with that of contemporary carbon. After the administration of 0.855 μc of hydrocortisone-4-C^{14}, the pooled carbon dioxide collections contained 20 ± 1 DPM per gram of carbon. This increase of 5 DPM per gram of carbon in the expired air represents excreta of radioactivity equivalent to 0.05 per cent of the administered hydrocortisone per day. It is unlikely that degradation of the A ring or, by implication, the entire steroid nucleus exceeds this amount.

DISCUSSION

The results of these studies reveal several new aspects of transport, distribution, and fate of the most important adrenal hormone in human subjects. It would be well to emphasize that the "tracer" dose, without carrier hormone, was well within the physiological range and in no sense an excessive or abnormal addition to the endogenous, glandular production of hydrocortisone. The "tracer" dose was 0.25 mg., introduced into the blood stream during a half-hour period. From the quantity of C_{19}-11 oxygenated steroids in normal urine and from the amount of these same compounds that can be recovered after the administration of exogenous hormone, it can be inferred that the human adrenal supplies about 30 mg. of hydrocortisone or similar steroids per day. The credibility of this estimate is supported by the fact that a totally adrenalectomized human, with minor supportive therapy such as desoxycorticosterone, can be maintained on approximately 25 mg. of hydrocortisone given by continuous intravenous infusion. Therefore, approximately 1 mg. of steroid per hour would be delivered into the circulation. The "tracer" dose of 0.25 mg. then represented approximately half the subject's hormone production during 30 minutes in which the hormone was administered, and was well within acceptable physiological limits. In the experiments where 100 mg. of carrier hydrocortisone was administered together with the labeled compound, the amount injected represented a relative excess of approximately 200 times the daily hormone requirement for that interval; the total amount administered was 400 times the tracer dose of the same hormone.

With these considerations in mind, it is possible to arrive at certain conclusions about both "utilization" and "tissue demand" for the adrenocortical hormone. If it is assumed that the 80 per cent of the 0.25 mg. "tracer" dose excreted during the first day represented disposition of an excess of hormone, and if it is assumed that only the 50 γ *possibly* remaining in the tissues after the first day represented that portion of the dose actually utilized in physiological mechanisms, it might be expected that all but 50 γ of the 100 mg. dose would be similarly excreted during the first day. Since this was not the case and in fact an essentially identical fraction of both dose levels was excreted during the first day by the same as well as by different subjects, the conclusion is warranted that widely differing quantities of hormone are handled

in a similar fashion, by mechanisms independent of tissue requirements. In order to subject this conclusion to a critical test, a totally adrenalectomized and oophorectomized patient was deprived of all exogenous hormone for two days preceding the administration of a 0.25 mg. dose of hydrocortisone-4-C^{14}. It would be expected, and it can be strongly supported from the other results of this study, that the tissues of this patient were depleted of all residual hormone to the fullest extent obtainable in a human subject. In addition, the 250 γ dose of hormone represented only half the normal requirement for the period of the experiment and even less of the total daily need. If tissue requirements or "demand" influenced the excretion of hormone or its metabolites, retention of hormone should have been maximal in this patient. However, the rate and the total quantity excreted under these conditions was almost identical with those of the other subjects. Subsequently, when the tissue requirements were altered in this subject by the continuous daily infusion of 100 mg. of hydrocortisone, an identical pattern of excretion of the labeled hormone once more was observed. It is noteworthy also that in both experiments in this subject, the blood levels of radioactivity were in the same range. Therefore, by the criteria used, there was no evidence for tissue "demand" and no difference was observed in the "utilization" of a large as compared with a small dose. Indeed, the total excretion of radioactive products derived from the hormone, as well as the rate at which this occurred, was remarkably constant in all of the patients studied. This constant pattern under experimental conditions deliberately chosen to represent physiological, pharmacological, and pathological extremes, suggests that the same metabolic pattern will be found in all human subjects studied in this manner.

In the living organism, endogenously produced or artificially administered hormone is subjected to at least two potentially competitive processes. In the one, a hormone with its unique chemical characteristics is needed in a biological system for the initiation or regulation of a series of biochemical reactions that in turn lead to the well-known physiological effects of an endocrine secretion. The hormone may or may not undergo certain chemical alterations as a result of participation in these reactions. It could be suggested that, whether as a necessary or purely incidental consequence of reaction in a cellular process, the hormone attached to a cellular constituent undergoes transformation as a result of which it becomes readily dissociated from the complex in which it had performed its function. This altered product is rapidly conjugated or further metabolized and speedily eliminated from the body. Since the metabolites are invariably less effective hormonal agents than their precursors, and since the conjugated metabolites are practically devoid of biological activity, the chemical transformation and conjugation of the hormone may be the means by which the organs or tissues influence the level of homeostasis. While this series of events is in process with one fraction of the hormone, other reactions *may* be in operation simultaneously. In these, the hormone may be metabolized, conjugated and eliminated from the site of these reactions without having exerted any biochemical or biological actions despite the fact that cortisone or hydrocortisone has been shown to influence the dynamic behavior of practically every tissue in the body (6). It may, nevertheless, be viewed as a type of inactivation mechanism, designed perhaps to prevent sudden fluctuations in the highly integrated performance of the whole organism. The contribution of each of the processes to the sum of the hormonal metabolites cannot be evaluated since the fraction of the hormone used for a specific biological action and the questionable portion that may be "non-specifically" transformed and eliminated are unknown. Differences may yet be found in the metabolites excreted after a "tracer" dose and after a larger amount. The urinary metabolites of hydrocortisone-4-C^{14} under the several conditions studied are at present being examined in an attempt to clarify these problems.

The findings that the peak excretion of radioactive products derived from hydrocortisone-4-C^{14} occurred during the second hour after intravenous administration indicate that the hormone had been retained in one or another of these intermediate metabolic processes before it appeared in the urine. The excretion in the urine during the first few hours of the study certainly represents other mechanisms than direct renal clearance of the unaltered hormone. If this only were involved,

the greatest excretion would have occurred immediately after the compound was given when the circulating levels were at a maximum. From the results, it is clear that hormone is removed from the circulation by processes which operate at a more rapid rate than excretion by the kidney. Analysis of blood specimens taken during the infusion when 50 per cent of the total dose of the hormone had been delivered into the circulation as well as at the conclusion of the infusion when the entire dose had been given, indicated that a large portion of the homone had been removed from the blood and either distributed in extracellular fluids or deposited in tissues or both. Calculations of the approximate amount of radioactivity distributed in extracellular fluid were made from the average values for extracellular fluid and the concentration of radioactivity in blood. At the end of the infusion from 44 to 135 per cent with an average value of 93 per cent could have been in this compartment, if the assumption that equilibrium exists is correct. The large variation is due in part at least to the relatively low precision in measurement of radioactivity in blood. In earlier animal experiments with tritium labeled cortisone, it was found that 70 per cent of an intravenous dose of the hormone could be found in the liver within 5 minutes after administration (7). At later time intervals, the amount in the liver declined so that uptake by the liver is probably a transitory process. If the observations in mice may be applied to man, it is likely that most of the hydrocortisone-4-C^{14} was removed from the circulation by the liver, and later reintroduced into the blood stream, probably in a chemically altered form that could then be excreted by the kidney. Additional evidence for this viewpoint is obtained from the data concerning the fraction of hormone excreted in the free as compared to the conjugated form. In the second hour following the infusion, from 81 to 96 per cent of excreted material was already in a conjugated form and by 6 hours, from 92 to 99 per cent was conjugated. The hormone had clearly been subjected to certain chemical reactions and alterations in the short interval between its administration and excretion of products representing the majority of the original material.

Regardless of the mechanisms responsible for rapid removal of hydrocortisone from the blood, in order to arrive at an explanation of the constant pattern of excretion observed in these studies, the following considerations are pertinent. The systems concerned with the localization, transformation, and conjugation of the hormone have a high capacity so that large as well as small doses are handled with equal facility. The altered hormone or the hormone metabolites after discharge from the site of transformation attain a concentration in the body fluids, including blood, in direct proportion to the amount of material initially administered. Since the capacity of the systems for conversion are not rate limiting, the concentration of radioactive hormone metabolites in the circulation is achieved at the same time and in direct proportion to the dose administered. These are now excreted by a renal mechanism that is independent of all factors except the concentration of these compounds in the blood passing through the kidney. Kidney excretion, therefore, would always constitute a constant fraction of the dose administered and the identical characteristics of the excretion curve in all the patients could be explained.

Some other observations on the role of the kidney in the total metabolism of hydrocortisone are pertinent. The adrenalectomized patient, subject F4, who received a tracer amount of hydrocortisone while all hormone therapy was withdrawn, showed the renal excretory changes characteristic of adrenal insufficiency. The volumes of the hourly urine specimens obtained during the course of the experiment were extremely small, contained a great deal of salt, and there was delay in the excretion of a water load. Despite this marked alteration in renal function, the quantitative aspects of the excretion of hormone metabolites was precisely that exhibited by normal subjects as well as by this same adrenalectomized patient when she was receiving 100 mg. of hydrocortisone daily by continuous intravenous infusion as replacement therapy. Reduction in urine flow to the extent observed in this patient was therefore not a limiting factor in the biological life-time of the hormone and its metabolites. Renal clearance, calculated from the average amount of radioactivity excreted per minute at the end of 2 hours and the blood radioactivity measured at that time, indicates that from 30 to 140 ml. of blood per minute were cleared of radioactivity. Here again the low precision of blood C^{14} measurements makes the estimate only an approximation. Nevertheless the

values are less than the anticipated glomerular filtration rate and it can be suggested that tubular secretion is not an important excretory mechanism for the metabolites of hydrocortisone.

One of the most sensitive and prompt responses to hydrocortisone whether artificially introduced or secreted by the gland under stimulation with adrenocorticotrophic hormone is the fall in level of the circulating eosinophiles. This occurs after about 4 hours during constant intravenous infusion while the other manifestations of the hormone such as nitrogen excretion, glycogen deposition and the like are observed only after a longer time. It is clear from the results reported that even the onset of a biological response is seen at a time when a sensible proportion of the total amount of hormone given has been completely eliminated from the body. In addition to this, it is highly likely that a major portion of the hormone still resident in tissues or body fluids had already undergone chemical alteration. It seems evident therefore that the chain of reactions initiated by the hormone demands a continued supply of small amounts of the compound for the expression of a physiological or pharmacological response.

The possibility remains that a very small portion of the total hormone administered is localized in particular structures intimately associated with the true hormonal function. That this is an unlikely possibility is strongly suggested from the fact that 93 per cent of the administered radioactivity was recovered in the urine and feces and no more was recovered from the large dose than from the tracer amount of hydrocortisone. It seems more probable that a small additional quantity of hormone metabolites had reached the gastrointestinal tract and will eventually be found in the feces; in addition some of the radioactivity may have been eliminated through the skin. In view of the many uncertainties associated with the missing 5 to 10 per cent of the hormone, it seems more reasonable to focus attention upon the larger fraction that has been recovered rather than to speculate about the questionable significance of the minor quantity. Similar considerations might be applied to the small sustained urinary elimination observed after the first 24 hours following introduction of the hormone. The rate of excretion of radioactivity during the first 24 hours was essentially constant in all subjects and could be characterized by a half-life time of 3.6 hours. After the first day, the rate of elimination was entirely different from this value. It seems quite reasonable to suppose that this latter radioactivity represents hydrocortisone which has been eliminated into the gastrointestinal tract, reabsorbed, further metabolized and perhaps again subjected to similar cycles prior to its appearance in the urine.

The striking analogy between the excretion of the metabolites of hydrocortisone and of testosterone is worthy of comment. Although the experimental procedure was not the same as that employed with the adrenal hormone, it was found that a similarly rapid excretion of the hormone metabolites occurred (8). Thus, 62 per cent of the total metabolites of testosterone recovered during the first day after an intravenous infusion lasting for 4 hours, was excreted during the period of infusion. Like the hydrocortisone metabolites, these urinary products were chemically altered and conjugated forms of the hormone administered. The similar fate of two steroid hormones with opposite biological properties presents a challenging problem in the correlation of urinary steroids with the metabolic processes under hormonal control.

Since negligible amounts of C^{14} appeared in the respiratory CO_2, it is apparent that no major destruction of the nucleus occurred. It is perhaps significant to note that a similar situation obtains with the nucleus of cholesterol (8) despite oxidative degradation of the side chain. Further studies of C-20 or C-21 labeled hydrocortisone would be of interest in this connection.

The urinary metabolites of the steroid hormones are used widely as a measure of adrenal function in man. While the facts and assumptions which underlie this measurement are almost certainly true, the inadequacy of the methods which have been applied to characterize adrenal function in man by these means must be emphasized. Many specific and non-specific determinations of certain functional groups in the cortisone and hydrocortisone molecule have been used as clinical indices of the performance of the adrenal gland in a variety of physiological or pathological conditions. A survey of these methods indicates that the majority account for but a fraction of the hormone administered. By inference they should account for only a fraction of the hormone produced by the

normally functioning adrenal gland. The present study clearly demonstrates that about 80 per cent of the hormone administered in either trace quantities or massive doses appeared in one form or another in the urine within the course of 24 hours. Indeed, most of the end products are excreted within a much shorter period of time. The characterization and definition of these various transformation products of the hormone in order to permit the development of satisfactory methods for their determination still remains a major task.

SUMMARY

The dynamics of the distribution and excretion of hydrocortisone-4-C^{14} has been studied in six experiments with four human subjects. Over 90 per cent of the radioactivity administered was excreted in the urine and feces in 72 hours. There was no evidence of *in vivo* breakdown of the hormone as determined from measurements of expired carbon dioxide. No radioactivity was found in the spinal fluid of one subject 8 hours after the intravenous injection of the hormone. Within 24 hours, from 70 to 80 per cent of the hormone was eliminated in the urine in the form of conjugated metabolites at a rate characterized by an average half-life of 3.6 hours. This rate and the cumulative excretion were independent of the amount of hormone injected and the physiologic or pathologic status of the subject. There was no apparent difference in the fate of a "tracer" dose administered to an adrenalectomized oophorectomized subject deprived of all therapy as compared with a "tracer" dose while the same subject was maintained on adequate intravenous hydrocortisone. The blood level of radioactivity was measured at various intervals during and after the infusion. The rapid metabolism of hydrocortisone was demonstrated by the finding that the unaltered hormone accounted for only 10 per cent of the blood radioactivity within 15 minutes after completion of the intravenous infusion.

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THE EFFECT OF BARBITURATES IN PATIENTS WITH LIVER DISEASE [1]

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Although sedatives are frequently required in the treatment of patients with liver disease, barbiturates are believed to be contraindicated in the face of impaired hepatic function. Three points are advanced in support of this belief: First, patients with advanced liver disease have remained comatose for long periods of time after receiving a barbiturate; second, animals after chloroform induced hepatic damage or partial liver extirpation have shown an increased sensitivity to anesthetic doses of barbiturates (1, 2); and third, animals are alleged to show a greater than normal retention of pentobarbital and barbital in their blood and tissues after chloroform anesthesia (3). These points of evidence, however, are far from conclusive. The natural progression of hepatic coma from a stage of restlessness to one of somnolence, and the spontaneous fluctuations that occur in the course of liver disease reduce the significance of temporal correlations in determining the part any single factor plays in causing hepatic coma. As far as animal experiments are concerned, they are not necessarily applicable to man, particularly if the type of liver damage and the barbiturate dosage are not comparable to the usual clinical situation. Finally, methods of barbiturate analysis are difficult and non-specific. Since the evidence incriminating the use of barbiturates in liver disease seemed circumstantial, a study to obtain direct evidence was undertaken. Three questions in particular seemed worthy of consideration:

1. Do barbiturates damage the liver itself?
2. Are patients with liver disease more sensitive to barbiturates because of impaired inactivation of these drugs by the damaged liver?
3. Are patients with liver disease more sensitive to barbiturates because of increased sensitivity of the nervous system?

Information bearing on these questions was sought by comparing the effect of barbiturates in control subjects and patients with liver disease. Pentobarbital (Nembutal®) was the barbiturate principally used because it is a popular drug and is said to be inactivated by the liver. The effects of both single doses and prolonged administration of barbiturate were observed.

EFFECTS OF SINGLE DOSES OF BARBITURATE

Material and methods.

Pentobarbital was given to 35 control subjects without liver or central nervous system disease and to 21 patients with liver disease. As indicated in Table I, all of these patients except one had signs and symptoms of advanced hepatic damage. Seventeen of these patients had cirrhosis with an alcoholic background but at the time of the acute studies were suffering neither from alcoholism nor its withdrawal symptoms. After preliminary studies showed that oral doses were well tolerated, intravenous administration (sodium pentobarbital) was used routinely in subsequent studies to eliminate the variables of intestinal absorption and to provide a sharp beginning point for both clinical and biochemical measurements. The drug was given either as a single injection during the course of one-half minute or as a constant intravenous infusion over a 30-minute period. Single injections were given 40 times to patients with liver disease and 35 times to control subjects. The doses varied from 30 to 240 mg., but in 75 per cent of the tests 100 to 120 mg. was used. Doses given to control and patient groups were comparable. Intravenous infusions containing 100 to 180 mg. of sodium pentobarbital in 120 ml. were given to 10 patients with liver disease and to seven control subjects.

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TABLE I
DIAGNOSES AND EVIDENCES OF HEPATIC DYSFUNCTION IN THE PATIENTS STUDIED

	Patient	Diagnosis	Esophageal Varices	Ascites	Albumin Gm./100ml plasma	Total Serum Bilirubin mg. %	Prothrombin Time % of normal	Formol Gel
PATIENTS GIVEN SINGLE DOSE OF BARBITURATE – ACUTE STUDIES								
1	C.R.	Laennec's Cirrhosis	NO	YES	2.1	6.8	26	not done
2	H.K.	"	"	"	2.7	18.8	75	"
3	M.C.	"	"	"	2.1	5.7	38	"
4	S.V.	"	"	"	2.3	2.3	50	"
5	J.N.	"	YES	"	2.5	1.6	45	"
6	E.D.	"	"	"	2.8	2.5	30	"
7	J.C.	"	NO	"	2.6	11.0	26	"
8	A.M.	"	not examined	"	1.1	25.5	27	"
9	H.C.	"	YES	"	1.9	1.4	30	"
10	F.M.	"	"	"	2.1	7.8	27	"
11	L.F.	"	"	"	2.8	0.5	30	"
12	J.F.	"	"	"	2.2	1.3	48	"
13	M.C.	"	"	"	2.0	2.7	44	"
14	C.S.	"	NO	"	1.7	2.1	65	"
15	J.H.	"	not examined	"	2.0	2.8	75	"
16	W.W.	"	"	NO	2.3	7.0	37	"
17	O.H.	"	NO	YES	2.8	1.8	70	"
18	G.W.	Hepatoma	YES	"	2.5	2.8	69	"
19	W.D.	"	NO	"	2.5	1.7	65	"
20	O.T.	Biliary Cirrhosis	YES	"	2.0	7.4	48	"
21	I.L.	Viral Hepatitis	NO	NO	4.4	0.8	70	"
PATIENTS GIVEN REPEATED DOSES OF BARBITURATE – PROLONGED STUDIES								
1	C.	Laennec's Cirrhosis	NO	YES	not done	3.3	35	4+
2	B.	"	"	"	"	10.8	28	4+
3	T.	"	YES	"	"	5.6	42	4+
4	M.	"	not examined	"	"	4.3	68	4+
5	C.	"	YES	"	"	1.5	42	4+
6	D.	"	NO	"	"	2.0	45	4+
7	C.	"	NO	"	"	12.2	39	4+
8	H.	"	YES	"	3.0	4.0	30	4+
9	C.	"	not examined	"	not done	0.7	36	4+
10	S.	"	"	"	"	43.0	61	4+

Evaluation of barbiturate effects was attempted by three general methods: clinical observation, liver function tests and measurement of pentobarbital blood levels. Since there are no established methods for measuring either clinical manifestations or pentobarbital blood levels that follow the intravenous injection of ordinary hypnotic doses of pentobarbital, the choice, development and adequacy of methods used by us are discussed in some detail.

Clinical observations.

When it appeared that the doses used in our study would not elicit the signs described as characteristic of acute and chronic barbiturate intoxication, a number of other neuropsychologic tests were investigated. The tests listed in Table II were performed before the barbiturate was given and at 5 to 30 minute intervals thereafter for one hour, or until all evidences of drug effect had disappeared. The most useful tests for following the effects of pentobarbital proved to be nystagmus, slurred speech, two-point discrimination and evaluation of changes in mood and levels of consciousness. Other tests were omitted when it became apparent that they were of little help in assessing barbiturate effects. The learning factor, varying effects of pentobarbital on mood, the degree of patient cooperation and differences in educational background made the interpretation of reading and mathematical tests difficult. No definite changes in temperature, pulse, respiration and blood pressure were noted in either patients or control subjects after barbiturates were given.

Intravenous administration of 60 mg. or less of sodium pentobarbital produced minimal changes, nystagmus on lateral gaze and slight variations in mood (euphoria, sense of "relaxation") being the usual effects of the drug. Barbiturate activity began within one-half minutes of injection and persisted for 15 to 30 minutes.

Larger intravenous doses of pentobarbital (100 to 180 mg.) intensified the nystagmus and changes in mood and induced other measurable effects. Nystagmus, as it increased, occurred in the vertical as well as horizontal plane and was accompanied frequently by blurred vision and occasionally by diplopia. Slurred speech or abnormally precise speech usually began in the first five minutes of the post-medication period and subsided during the following 20 minutes. Impaired two-point discrimination was found to correlate well with the over-all pentobarbital effect. Usually the threshold for two-point discrimination increased by 50 to 100 per cent after injecting the drug; this increase was maximal during the first 15 minutes and seldom persisted for more than 30 minutes.

The larger doses of pentobarbital also increased the degree of euphoria. The subjects were frequently more garrulous and likened the effect to that produced by alcohol. Within 15 minutes,

TABLE II

Clinical observations made on the effects of single doses of barbiturate

Tests	Times performed: Patients with liver disease	Times performed: Control subjects
Nystagmus	20	28
Slurred speech	15	28
Two-point discrimination	15	28
Changes in mood and level of consciousness	40	35
Deep-tendon reflexes	22	18
Finger to nose	29	16
Mathematical problems	18	7
Reading test	9	6
Corneal reflex	9	6
Temperature, pulse, respirations	9	6

TABLE IIIa

The effect of pentobarbital, 100–120 mg. I.V. as a single injection, on 28 control subjects

Pt.	Age	Diagnosis	Test	Degree of drug effect—0 to 4 Duration of drug effect in minutes 0	15	30	45	60	75	90	Sum of effect
			Given in 2 ml.								
G. B.	74	Hypertensive cardiovascular disease	Nystagmus	0	2	2	1	0	0	0	5
			2-pt. discrim.	0	2	2	0	0	0	0	4
			Slurred speech	0	3	2	0	0	0	0	5
			Change in mood	0	3	3	3	3	2	1	9
										Barbiturate index	23
S. M.	70	Generalized arteriosclerosis	Nystagmus	0	2	2	1	0	0	0	5
			2-pt. discrim.	0	2	1	0	0	0	0	4
			Slurred speech	0	2	2	1	0	0	0	5
			Change in mood	0	3	2	1	1	0	0	7
										Barbiturate index	21
E. N.	42	Essential hypertension	Nystagmus	0	1	1	0	0	0	0	3
			2-pt. discrim.	0	1	0	0	0	0	0	2
			Slurred speech	0	2	1	0	0	0	0	4
			Change in mood	0	1	1	1	0	0	0	4
										Barbiturate index	13
D. B.	59	Bronchial asthma	Nystagmus	0	2	1	0	0	0	0	4
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	1	0	0	0	0	0	2
			Change in mood	0	2	1	1	0	0	0	5
										Barbiturate index	11
F. C.	19	Duodenal ulcer	Nystagmus	0	2	1	0	0	0	0	4
			2-pt. discrim.	0	1	0	0	0	0	0	2
			Slurred speech	0	0	1	0	0	0	0	2
			Change in mood	0	1	0	0	0	0	0	2
										Barbiturate index	10
M. M.	55	Essential hypertension	Nystagmus	0	3	2	1	0	0	0	6
			2-pt. discrim.	0	2	1	1	1	0	0	6
			Slurred speech	0	1	1	0	0	0	0	3
			Change in mood	0	1	1	0	0	0	0	3
										Barbiturate index	18
E. R.	40	No disease	Nystagmus	0	2	1	1	0	0	0	5
			2-pt. discrim.	0	2	1	0	0	0	0	4
			Slurred speech	0	1	0	0	0	0	0	2
			Change in mood	0	1	0	0	0	0	0	2
										Barbiturate index	13
D. C.	21	Erythema nodosum	Nystagmus	0	2	1	1	0	0	0	5
			2-pt. discrim.	0	1	0	0	0	0	0	2
			Slurred speech	0	1	0	0	0	0	0	2
			Change in mood	0	2	1	0	0	0	0	4
										Barbiturate index	13
L. G.	66	Duodenal ulcer	Nystagmus	0	2	1	1	0	0	0	5
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	1	0	0	0	0	0	2
			Change in mood	0	2	1	1	0	0	0	5
										Barbiturate index	12

TABLE IIIa—*Continued*

Pt.	Age	Diagnosis	Test	Degree of drug effect—0 to 4 Duration of drug effect in minutes 0	15	30	45	60	75	90	Sum of effect
			Given in 2 ml.								
M. H.	54	Post-chole-cystectomy	Nystagmus	0	3	2	1	0	0	0	6
			2-pt. discrim.	0	0	1	0	0	0	0	2
			Slurred speech	0	3	0	0	0	0	0	4
			Change in mood	0	2	1	1	0	0	0	5
										Barbiturate index	17
E. V.	58	Anxiety state	Nystagmus	0	1	0	0	0	0	0	2
			2-pt. discrim.	0	2	0	0	0	0	0	3
			Slurred speech	0	0	0	0	0	0	0	0
			Change in mood	0	1	0	0	0	0	0	2
										Barbiturate index	7
L. T.	27	Essential hypertension	Nystagmus	0	2	1	0	0	0	0	4
			2-pt. discrim.	0	2	1	0	0	0	0	4
			Slurred speech	0	1	0	0	0	0	0	2
			Change in mood	0	2	1	0	0	0	0	4
										Barbiturate index	14
A. H.	64	Rheumatoid arthritis	Nystagmus	0	3	3	2	2	0	0	7
			2-pt. discrim.	0	1	1	0	0	0	0	3
			Slurred speech	0	1	0	0	0	0	0	2
			Change in mood	0	2	1	0	2	0	0	5
										Barbiturate index	17
C. J.	38	Pulmonary infarction	Nystagmus	0	2	1	1	1	0	0	6
			2-pt. discrim.	0	2	1	0	0	0	0	4
			Slurred speech	0	2	1	1	0	0	0	5
			Change in mood	0	1	1	1	0	0	0	4
										Barbiturate index	19
E. B.	74	Cholelithiasis	Nystagmus	0	2	1	0	0	0	0	4
			2-pt. discrim.	0	2	1	0	0	0	0	4
			Slurred speech	0	4	0	0	0	0	0	5
			Change in mood	0	2	1	0	3	0	0	6
										Barbiturate index	19
R. C.	64	Obesity	Nystagmus	0	3	2	1	0	0	0	6
			2-pt. discrim.	0	1	2	0	0	0	0	4
			Slurred speech	0	1	0	0	0	0	0	2
			Change in mood	0	2	2	1	0	0	0	5
										Barbiturate index	17
D. B.	40	Post-enceph. Parkinsonism	Nystagmus	0	3	2	2	2	2	2	9
			2-pt. discrim.	0	2	1	0	0	0	0	4
			Slurred speech	0	2	1	0	0	0	0	4
			Change in mood	0	3	2	2	2	2	2	9
										Barbiturate index	26
M. C.	66	Generalized arteriosclerosis	Nystagmus	0	3	3	2	1	1	1	9
			2-pt. discrim.	0	1	1	0	0	0	0	3
			Slurred speech	0	3	2	1	0	0	0	6
			Change in mood	0	3	2	2	1	0	0	7
										Barbiturate index	25
			Given in 120 ml. infusion								
W. B.	65	Duodenal ulcer	Nystagmus	0	2	3	3	2	2	1	9
			2-pt. discrim.	0	0	1	1	0	0	0	3
			Slurred speech	0	1	2	1	0	0	0	5
			Change in mood	0	0	2	3	2	1	0	7
										Barbiturate index	24

TABLE IIIa—*Continued*

Pt.	Age	Diagnosis	Test	Degree of drug effect—0 to 4 Duration of drug effect in minutes 0	15	30	45	60	75	90	Sum of effect
			Given in 120 ml. infusion								
R. D.	31	Neurofibromatosis	Nystagmus	0	0	2	2	1	0	0	5
			2-pt. discrim.	0	0	1	1	0	0	0	3
			Slurred speech	0	0	0	0	0	0	0	0
			Change in mood	0	0	2	1	0	0	0	4
									Barbiturate index		12
J. S.	28	No disease	Nystagmus	0	0	0	1	1	0	0	3
			2-pt. discrim.	0	1	2	1	1	0	0	6
			Slurred speech	0	0	0	0	0	0	0	0
			Change in mood	0	0	0	2	1	0	0	4
									Barbiturate index		13
J. C.	59	? Pulmonary tuberculosis	Nystagmus	0	1	3	2	1	0	0	7
			2-pt. discrim.	0	0	2	2	0	0	0	4
			Slurred speech	0	0	2	1	0	0	0	4
			Change in mood	0	0	1	3	1	0	0	6
									Barbiturate index		21
H. B.	30	Anxiety state	Nystagmus	0	1	2	3	1	0	0	7
			2-pt. discrim.	0	0	1	0	0	0	0	2
			Slurred speech	0	0	2	1	0	0	0	4
			Change in mood	0	0	1	0	0	0	0	2
									Barbiturate index		15
N. P.	76	Generalized arteriosclerosis	Nystagmus	0	1	2	2	1	0	0	6
			2-pt. discrim.	0	0	1	1	0	0	0	3
			Slurred speech	0	0	1	1	0	0	0	3
			Change in mood	0	0	3	3	1	0	0	6
									Barbiturate index		18
A. B.	56	Hypertensive cardiovascular disease	Nystagmus	0	3	3	3	2	0	0	7
			2-pt. discrim.	0	0	1	0	0	0	0	2
			Slurred speech	0	0	3	1	0	0	0	5
			Change in mood	0	1	3	1	0	0	0	6
									Barbiturate index		20
T. S.	32	Pharyngitis	Nystagmus	0	1	3	2	1	0	0	7
			2-pt. discrim.	0	0	2	1	0	0	0	4
			Slurred speech	0	0	2	1	0	0	0	4
			Change in mood	0	0	2	3	1	0	0	6
									Barbiturate index		21
J. E.	42	Duodenal ulcer	Nystagmus	0	1	3	3	2	0	0	7
			2-pt. discrim.	0	0	1	0	0	0	0	2
			Slurred speech	0	0	1	2	1	0	0	5
			Change in mood	0	0	1	2	3	0	0	6
									Barbiturate index		20
F. C.	19	Duodenal ulcer	Nystagmus	0	1	3	0	0	0	0	5
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	1	1	0	0	0	0	3
			Change in mood	0	0	1	0	0	0	0	2
									Barbiturate index		10

TABLE IIIb

The effect of pentobarbital, 100–120 mg. I.V. as a single injection, on 15 patients with liver disease

Pt.	Age	Diagnosis	Test	Degree of drug effect—0 to 4 Duration of drug effect in minutes 0	15	30	45	60	75	90	Sum of effect
			Given in 2.0 ml.								
G. W.	72	Hemochromatosis Cirrhosis Hepatoma	Nystagmus	0	2	1	0	0	0	0	4
			2-pt. discrim.	0	3	2	0	0	0	0	5
			Slurred speech	0	3	0	0	0	0	0	4
			Change in mood	0	2	3	3	3	2	1	9
										Barbiturate index	22
O. T.	47	Biliary cirrhosis	Nystagmus	0	2	2	1	0	0	0	5
			2-pt. discrim.	0	1	1	1	0	0	0	4
			Slurred speech	0	1	0	0	0	0	0	2
			Change in mood	0	2	1	0	0	0	0	4
										Barbiturate index	15
C. R.	49	Laennec's cirrhosis	Nystagmus	0	1	1	0	0	0	0	3
			2-pt. discrim.	0	3	2	1	1	0	0	7
			Slurred speech	0	3	0	0	0	0	0	4
			Change in mood	0	1	0	0	0	0	0	2
										Barbiturate index	16
			Given in 120 ml. infusion								
J. F.	45	Laennec's cirrhosis	Nystagmus	0	1	2	2	0	0	0	5
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	1	1	0	0	0	0	3
			Change in mood	0	1	2	2	0	0	0	5
										Barbiturate index	13
J. L.	35	Viral hepatitis	Nystagmus	0	0	1	1	0	0	0	3
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	0	2	2	1	0	0	5
			Change in mood	0	0	1	0	0	0	0	2
										Barbiturate index	10
O. H.	44	Laennec's cirrhosis	Nystagmus	0	1	3	2	1	0	0	7
			2-pt. discrim.	0	0	1	1	1	0	0	4
			Slurred speech	0	1	2	1	0	0	0	5
			Change in mood	0	1	2	1	0	0	0	5
										Barbiturate index	21
W. W.	51	Laennec's cirrhosis	Nystagmus	0	0	2	1	1	0	0	5
			2-pt. discrim.	0	0	1	0	0	0	0	2
			Slurred speech	0	0	2	1	0	0	0	4
			Change in mood	0	0	1	2	0	0	0	4
										Barbiturate index	15
C. S.	48	Laennec's cirrhosis	Nystagmus	0	0	1	1	0	0	0	3
			2-pt. discrim.	0	0	1	1	0	0	0	3
			Slurred speech	0	0	0	0	0	0	0	0
			Change in mood	0	0	1	1	0	0	0	3
										Barbiturate index	9
W. W.	52	Laennec's cirrhosis	Nystagmus	0	1	1	0	0	0	0	3
			2-pt. discrim.	0	1	1	0	0	0	0	3
			Slurred speech	0	0	1	1	0	0	0	3
			Change in mood	0	1	2	1	0	0	0	5
										Barbiturate index	14

TABLE IIIb—*Continued*

Pt.	Age	Diagnosis	Test	Degree of drug effect—0 to 4 Duration of drug effect in minutes 0	15	30	45	60	75	90	Sum of effect
			Given in 120 ml. infusion								
H. K.	43	Laennec's cirrhosis	Nystagmus	0	0	2	2	1	0	0	5
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	0	1	1	0	0	0	3
			Change in mood	0	1	2	1	0	0	0	5
									Barbiturate index		13
J. H.	65	Laennec's cirrhosis	Nystagmus	0	1	2	2	1	0	0	6
			2-pt. discrim.	0	0	1	1	0	0	0	3
			Slurred speech	0	0	2	2	1	0	0	5
			Change in mood	0	0	2	1	1	1	0	6
									Barbiturate index		20
J. F.	45	Laennec's cirrhosis	Nystagmus	0	1	2	2	1	0	0	6
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	0	0	0	0	0	0	0
			Change in mood	0	0	1	2	1	0	0	5
									Barbiturate index		11
M. C.	46	Laennec's cirrhosis	Nystagmus	0	0	1	2	0	0	0	4
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	0	1	2	0	0	0	4
			Change in mood	0	0	2	1	1	0	0	5
									Barbiturate index		13
J. H.	65	Laennec's cirrhosis	Nystagmus	0	1	2	1	0	0	0	5
			2-pt. discrim.	0	0	1	1	0	0	0	3
			Slurred speech	0	0	2	2	1	0	0	5
			Change in mood	0	0	1	1	1	0	0	4
									Barbiturate index		17
M. C.	36	Laennec's cirrhosis	Nystagmus	0	0	1	1	0	0	0	3
			2-pt. discrim.	0	0	0	0	0	0	0	0
			Slurred speech	0	0	1	1	1	0	0	4
			Change in mood	0	0	1	1	0	0	0	3
									Barbiturate index		10

however, euphoric symptoms tended to subside and drowsiness supervened. The drowsiness, which was of variable intensity, persisted longer than any other barbiturate effect. After two hours, however, all evidences of barbiturate activity disappeared. The subjects felt as they had before barbiturate injection, but two to four hours later a recurrent drowsiness overwhelmed many control subjects as well as patients with liver disease.

When constant infusions of sodium pentobarbital (4 to 6 mg. per minute for a total of 100 to 180 mg.) were used, each sign of barbiturate activity was checked for time of onset, rate of increase, peak effect attained and disappearance rate after completion of the infusion. A regular pattern of development and subsidence of barbiturate effect was noted and found to correspond with the amount of barbiturate infused. This parallelism between the amount infused and the development of nystagmus, slurred speech and impaired two-point discrimination supports the reliability of these signs as measures of the clinical response to intravenous barbiturates.

In an effort to quantitate pentobarbital effects and to facilitate the comparison of patient and control groups, an index of barbiturate activity was devised. Each of the four signs used to assess barbiturate activity was expressed numerically by adding its greatest intensity (rated on a scale of 1 to 4) and duration (1 unit for each 15 min.). The numbers representing each sign were then added to obtain the over-all index of barbiturate activity.

TABLE IV

Comparison of pentobarbital effects in control subjects and patients with liver disease

Age groups	Average barbiturate index	
	Control subjects	Patients with liver disease
20–40 yrs.	14	10
40–60 yrs.	16.6	13.9
over 60 yrs.	20.6	19.3

The 28 control subjects on whom all the major signs of barbiturate activity were checked are recorded in Table IIIa, the 15 patients with liver disease in Table IIIb.

The average index of barbiturate activity was calculated for different ages in both the control and patient groups (Table IV). Although individual doses varied from 100 to 120 mg., the average amount of barbiturate used in each group was comparable. The data indicate that patients with liver disease are no more affected by pentobarbital than are control subjects, but that increased sensitivity to this drug exists in older people irrespective of their hepatic function.

Liver function tests.

Serial liver function tests were carried out before and after injection of pentobarbital, two to four tests being performed at 15 to 30 minute intervals for one to two hours following injection. Since the number of different serial tests possible in any one patient is limited, no more than a pilot run was made with tests which were not expected to change suddenly in response to barbiturate administration. The number of serial tests done in patients with liver disease (L) and in control subjects (C) is indicated parenthetically after the name of each test: serum bilirubin (L-21, C-14), bromsulfalein clearance (L-23, C-9), thymol turbidity and flocculation (L-12, C-11), cholinesterase (L-10, C-12) (4), alkaline phosphatase (L-8, C-6) and cephalin cholesterol flocculation (L-5, C-5).

The injection of sodium pentobarbital did not affect results of the above tests. The average change of the serum bilirubin concentration in the patients with liver disease was, for example, only 0.02 mg. per 100 ml. The cholinesterase activity has been shown to be a sensitive index of hepatic function (5) and has been reported depressed after prolonged use of barbiturates (6), but in the acute tests reported here the pre- and post-barbiturate enzyme activity was virtually identical. When constant intravenous infusions of BSP are given, the rate of removal of this dye from the blood can be expressed as BSP clearance.[8] This test, which is fairly sensitive to gross changes in hepato-cellular activity or hepatic blood flow, was not affected for one hour after the injection of sodium pentobarbital.

Hepatic venous catheterization was performed successfully in two patients with chronic and three patients with early liver disease. Hepatic blood flow was estimated by the BSP method (7) and arterial-hepatic venous oxygen differences were measured. Intravenous injections of 120 mg. of sodium pentobarbital did not affect these measurements in any obvious manner.

Measurement of pentobarbital in blood.

Measurement of pentobarbital levels in serum was assayed in the hope of determining the effect of liver disease on these levels. The spectrophotometric method of Walker, Fisher and McHugh (8) was adopted for this purpose. Like other methods depending upon the absorption of ultraviolet light (235 to 240 mμ) by barbiturates, this technique is adequate but not entirely satisfactory. A number of interfering substances may appear in the blood, and the possibility that pharmacologically inactive forms of barbiturate may contribute to absorption at 240 mμ cannot be excluded.

In preliminary tests, recoveries of pentobarbital in known concentrations of 1 and 2 mg. per 100 ml. of serum were satisfactory. Storage of either serum or its protein-free filtrate at ice-box temperatures for three days did not impair this recovery. On the other hand, serum known to be free of barbiturates often gave readings up to 0.3 mg. per 100 ml. False readings up to 0.1 mg. per 100 ml. were obtained even in fasting subjects. When heparin was used, as in the measurement

[8] When an intravenous infusion of BSP is given at such a rate that the serum concentration of dye remains constant, BSP clearance (ml. per min.) =

$$\frac{\text{rate of BSP infusion in mg. per min.}}{\text{conc. of BSP in serum in mg. per ml.}}$$

In healthy young men, BSP clearance averages about 500 ml. per min.

of hepatic blood flow, satisfactory readings of barbiturate levels could not be obtained.

To compare the rate of disappearance of pentobarbital from the blood of healthy subjects and those with liver disease, serum levels were measured at 1 to 15 minute intervals for one hour after single intravenous doses. In both groups of subjects, erratic and exceedingly low values— usually between 0.2 and 0.4 mg. per 100 ml.—were obtained, even within 10 minutes of giving 120 mg. intravenously. In view of the satisfactory *in vitro* recoveries, we could only surmise that pentobarbital leaves the intra-vascular fluid compartment rapidly. This view agrees with the report of Brodie and his associates (9), who used anesthetic doses of pentobarbital in normal subjects, and noted an abrupt drop in plasma pentobarbital levels during the four hours following a single injection of the drug.

Since concentrations of pentobarbital in the serum after injection of clinical doses appeared to be erratic and low, and since measurement of the drug at low levels was invalidated by false readings, a new technique for comparing the rate of pentobarbital removal from the blood of normal subjects and liver patients was necessary. For this reason, constant infusions of sodium pentobarbital were given to seven control subjects and 10 patients with liver disease at the rate of 4 to 6 mg. per minute for 30 minutes. This method provided measurable drug levels in the serum, the amount of interfering substances being determined in blood taken immediately before the barbiturate infusion. The barbiturate levels obtained during the infusion were considered to represent the balance achieved between the infusion rate and removal mechanisms.

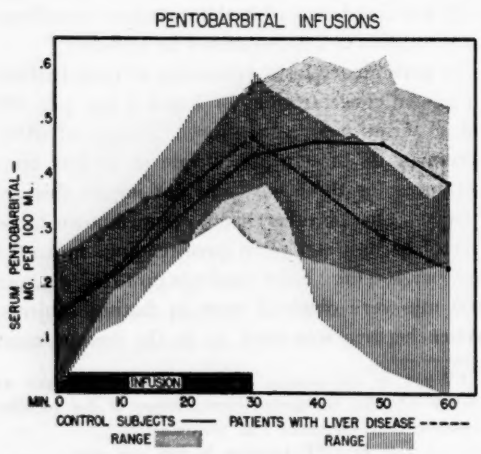

FIG. 1. SERUM PENTOBARBITAL CONCENTRATIONS DURING AND AFTER A CONSTANT INFUSION OF THE DRUG
The infusion ran from 0 through 30 minutes.

As shown in Figure 1, the removal of pentobarbital from the blood proceeded at an equal rate in seven control subjects and 10 patients with severe liver disease. On completion of the barbiturate infusion, pentobarbital blood levels appeared to decrease more rapidly in the liver patients than in control subjects. This may be due in part to rapid diffusion of pentobarbital from blood into ascitic fluid. Four observations made on this point showed pentobarbital levels in blood and ascitic fluid to be similar. Since our results probably reflect both the distribution and metabolism of pentobarbital, their significance is limited. It appears however, that the over-all handling of pentobarbital within one hour of its intravenous administration is not impaired by liver disease.

EFFECTS OF PROLONGED BARBITURATE THERAPY

Ten males with chronic cirrhosis (Table I) were given barbiturates over a prolonged period of time. Three of these men received the drug twice, making a total of 13 courses. Their ages ranged from 32 to 58, and all gave histories of alcohol ingestion and poor nutrition over many years. It is noteworthy that all these patients evidenced neurologic or psychologic abnormalities before receiving any barbiturates. Alcoholism rather than hepatic disease probably accounted for the prevalence of insomnia, tremors, nervousness, seizures and peripheral neuritis.

Two patients (three courses) received barbiturate therapy because they required sedation. In these, a pre-therapy baseline study was not possible. The remaining patients were followed for periods of 1 to 12 weeks before barbiturates were given. The total daily doses of pentobarbital were 50 mg. in two trials, 100 mg. in eight, and 250 mg. in one trial. Phenobarbital, 90 and 120 mg. daily, was given in two instances. In 12 trials, the drugs were given daily for periods varying from 11 to 41 days. The other trial (Case 1) was stopped after

five and one-half days because an untoward reaction was suspected.

Daily evaluation of the patient's mental status was made by the same observer, and frequent checks were made with ward physicians, nurses and neighboring patients. In only one case was there any deterioration of the patient's mental status during medication with barbiturates, and, as reported below (Case 1), the attending circumstances were quite complex. During the other 12 courses, the patients either were relieved of their nervous symptoms or, if asymptomatic, evidenced no impairment of their baseline intellectual level.

Since all of the patients suffered from advanced and chronic cirrhosis, obvious physical signs of liver disease persisted throughout their hospital stay. Some signs, such as jaundice and ascites, however, subsided sufficiently that the 10 cases could be discharged as moderately to markedly improved. Excepting the first trial in Case 1, the gradual incline of improvement, as evidenced by the disappearance of jaundice, ascites or both, was not flattened or reversed during the period of barbiturate administration.

Cephalin cholesterol flocculation, bromsulfalein retention, serum bilirubin, prothrombin time, total protein and formol gel reaction were measured every 7 to 10 days. Cholinesterase activity in the serum was determined serially in seven patients. On the whole, the tests reflecting protein metabolism did not change significantly during the period of hospitalization, but serum bilirubin dropped, bromsulfalein retention lessened, or cholinesterase activity increased as the patients grew better. Irrespective of its trend, none of the tests appeared to be affected by the use of barbiturates.

Electroencephalograms were obtained in four patients during barbiturate therapy and were compared with control records obtained before and after the therapy period. Bursts of bilateral, low-voltage fast activity, of variable duration and more prominent over the frontal lobes, were seen in two of the patients while on pentobarbital. Similar effects have been ascribed to barbiturates in patients without liver disease (10).

Psychological examinations of four of the patients were undertaken by a psychologist. Simple tests of intellectual operations were administered during control periods and during barbiturate exhibition. In general the patients performed within normal limits on all the tasks and at all times. Although the smallness of the sample precludes definite conclusions, the tentative impression was gained that test scores obtained were not affected by the intake of barbiturates.

Three of the patients were comatose at one point or another during their hospital stay. Their cases are reported briefly to illustrate two points: (a) when coma occurs in a cirrhotic patient receiving sedation, the responsibility of the sedative is not easily assessed; and (b), patients who suffer hepatic coma because of severe cirrhosis may tolerate pentobarbital once their coma has subsided.

Case 1. A 38-year old male, an alcoholic of long standing, was admitted because of liver disease with marked ascites and peripheral edema. He improved slowly, but because of an accidental increase in salt intake, a paracentesis yielding 9500 ml. was required one month after admission. The day of the tap, his serum sodium was estimated to be 130 mEq. per L. as calculated on the basis of serum chloride and bicarbonate values (11). Thirty-six hours later he was put on pentobarbital, 50 mg. twice daily. During the next 72 hours it was apparent that the patient was not doing well. He complained of lassitude, took to his bed, and frequently was found dozing. At this time his estimated serum sodium was 116 mEq. per L. Pentobarbital was stopped on the sixth day, but the patient continued to look ill and feel exhausted for another three weeks. Subsequently the patient improved, and two months after the tap received pentobarbital, 50 mg. twice daily, for 23 days without ill effect.

Case 2. A 51-year old patient with cirrhosis underwent subtotal gastric resection for pyloric obstruction. Postoperatively jaundice and then ascites appeared requiring paracentesis on the 13th postoperative day. For the next four days the paracentesis wound drained profusely, the patient losing a total of 27 pounds. For 10 days he had received 1.0 Gm. chloral hydrate for insomnia, but on the 15th and 16th postoperative nights he required 2.0 Gm. The morning of the 17th day he appeared confused, unresponsive, and very weak; the clinical impression was hepatic coma. His serum sodium, determined by flame photometry, was 110 mEq. per L. After hypertonic saline was given, his mental status ameliorated but his serum sodium was no more than 114 mEq. per L., and his weakness persisted. Three weeks later, the patient had improved somewhat, the serum sodium being 128 mEq. per L. As he still complained of insomnia, he was given pentobarbital, 100 mg. at bedtime, which he took for three weeks with relief of symptoms.

Case 3. A 58-year old man with cirrhosis was admitted with ascites. During the first two weeks he was anorectic and lethargic. He slept poorly at night and dozed in the daytime. During this phase he was given 25 mg. of

pentobarbital one morning and slept more than usual that day, although he could always be awakened easily. In the subsequent weeks, the patient improved slowly. One month after admission he received pentobarbital, 50 mg. at bed time, for 11 days without difficulty. He was discharged two months after admission but was readmitted three weeks later because of hematemesis. He was confused and lethargic on admission, and although sedation was not given, his clinical picture changed from one of restless confusion to progressive unresponsiveness. For 48 hours he reacted only to painful stimuli. His hemoglobin fell from 12.4 to 10.4 Gm., but the patient was never in shock. After remaining in coma for two days, the patient suddenly recovered and six days later received pentobarbital, 50 mg. twice daily, for 14 days without any evidence of depression of consciousness.

Pentobarbital was blamed so readily as being the primary cause of drowsiness and weakness in Case 1 that the possible role of hyponatremia subsequent to paracentesis was ignored. The sequence of events in Case 2, however, lead to re-evaluation of Case 1 with calculation in retrospect of approximate serum sodium values. In both cases the comatose state was preceded by a paracentesis which may have depleted the body's sodium store considerably, particularly since neither patient had peripheral edema to serve as a reservoir of electrolytes (12). Thus hyponatremia as well as the possible bad effects of pentobarbital or chloral hydrate must be weighed as a cause of the mental symptoms in Cases 1 and 2.

In Case 3, a brief but pronounced bout of coma occurred following a moderate hematemesis. The ability of this patient to tolerate the usual sedative dose of pentobarbital within six days of recovery from hepatic coma is noteworthy.

DISCUSSION

At the beginning of this study, the dangers of administering barbiturates to patients with liver disease loomed so vividly in our minds that doses of 15–30 mg. were used with considerable hesitation. When the expected ill effects failed to materialize, the dose was increased until it appeared that standard hypnotic doses were well tolerated, even by patients with severe cirrhosis. These findings, however, should not be used to justify an indiscriminate exhibition of barbiturate in hepatic disease; rather they may serve as a basis for examining the questions proposed in the introductory paragraphs.

1. Are barbiturates injurious to the liver? Since neither clinical nor biochemical impairment of hepatic function was evident following the use of barbiturates, it appears that the hepatotoxic potentialities of these agents have been overemphasized. The effect of barbiturates on several discrete hepatic functions was not studied, but the broad criterion afforded by the patient's clinical course did not inculpate pentobarbital as harmful to hepatic function.

2. Are barbiturates dangerous to patients with liver disease because hepatic inactivation of these drugs is impaired? In spite of the contradictory evidence obtained by subjecting animals with surgically extirpated or severely damaged livers to barbiturate narcosis, we could find no clinical indications that *hypnotic* doses of pentobarbital exert a deeper or more prolonged effect in patients with liver disease than in control subjects. Moreover, removal from the blood of barbiturates (or some degradation products) was not grossly different in the two groups. A delayed inactivation of barbiturates by patients with liver disease has therefore not been demonstrated by our studies.

3. Are barbiturates harmful to patients with liver disease because of increased sensitivity of the central nervous system? If the mental faculties of a cirrhotic patient are clouded by the unidentified metabolic factors of incipient hepatic coma, it seems reasonable to assume that the action of any central nervous system depressant will be at least additive. The additional effect of the sedative is, however, hard to assess, for the nervous manifestations of severe liver disease often pass spontaneously from restlessness to coma whether or not sedatives are given. Failure to recognize this natural progression leads to statements that barbiturates are particularly dangerous for the cirrhotic patient with bleeding varices. In these cases, a small dose of barbiturate given during the restless phase is often blamed for the ensuing coma, but this coma is usually the sequel of severe blood loss, not of sedation.

The possibility remains that patients on the verge of coma are actually hypersensitive to sedatives. If this is the case, the hypersensitivity appears to be a transient state conditioned by the metabolic abnormalities of hepatic coma. If hypersensitivity were determined by the structural

cerebral damage often found in chronic hepatic disorders (13), a constant barbiturate effect might be expected whether or not coma were impending. This does not seem to be the case. For example, the cirrhotic patients whose courses are briefly described tolerated barbiturates very adequately within two months of recovery from comatose states. Furthermore, our liver patients, as a group, were less sensitive to pentobarbital than elderly control subjects.

Current therapeutic practice favors the use of chloral hydrate or paraldehyde for the sedation of hepatic disease, a belief epitomized by Ravdin's enthusiastic statement "chloral hydrate has never been shown to cause hepatic injury" (14). To our knowledge, however, clinical studies showing either that other sedatives (*e.g.*, barbiturates) do harm the liver, or that chloral really is innocuous in human liver disease, do not exist.

Many patients with liver disease do not require sedation. Others who are confused, irrational, or restless require sedation desperately, but barbiturates, and other sedatives as well, may merely intensify the coma which usually succeeds upon the restless phase of liver failure. On the other hand, we suggest that pentobarbital may be used cautiously but without apprehension for allaying such minor nervous symptoms as insomnia, anxiety and tremors in patients with liver disease who do not exhibit the premonitory signs of coma.

SUMMARY

Pentobarbital in hypnotic doses was given to patients with severe liver disease and to control subjects. The effects of the drug were studied by clinical observations, liver function tests, and measurement of barbiturate blood levels.

No evidence was obtained that patients with liver disease were more sensitive to single injections of pentobarbital, or that the early removal of the drug from the blood of these patients was delayed.

Prolonged administration of pentobarbital to patients with decompensated liver disease did not appear to retard recovery in 12 of 13 trials.

The use of sedatives in patients verging on hepatic coma is not recommended, but the specific dangers ascribed to barbiturates in patients with liver disease appear to have been overemphasized.

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CONSIDERATIONS ON THE UPTAKE OF CARBON MONOXIDE BY THE LUNGS[1]

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In the course of investigations upon the disappearance of CO from the alveolar gas during breathholding following a single inspiration of a gas containing CO (1) we found it necessary to consider the effect of variations of this process in the different alveoli upon the behavior of the lung as a whole. The following discussion contains the more useful of these considerations, first for circumstances during breathholding (A) and secondly for circumstances during the steady state breathing of a gas mixture containing CO (B). Section C discusses the effect of significant amounts of COHb in the mixed venous blood upon the rate of CO uptake from the lungs, knowledge which was necessary in interpreting our results in experiments on heavy smokers. Section D considers the build-up of alveolar CO concentration, as the subject starts breathing a mixture containing CO. In other words, it describes the washout of the alveolar gases and achievement of the so called "steady state" condition before the mixed venous blood COHb concentration rises significantly. The final section E is a brief discussion of the build-up of COHb in the blood when the subject breathes CO for such long periods of time, that the processes covered in the earlier sections can be considered instantaneous.

A. *The Relation between the Mixed Expired Alveolar CO Tension and Time after a Single Inspiration of a Gas Containing CO*

In 1909, Krogh and Krogh (2) described the disappearance of CO from alveolar gas during a period of breathholding following a single inspiration of gas containing CO by the equation

$$F_A = F_{A_0} \exp\left(-\frac{DPbt}{V_A}\right), \qquad (1)$$

where F_A is alveolar concentration of CO (dry) at time t, F_{A_0} is alveolar concentration (dry) at time zero, "exp" is e, the base of the natural logarithms raised to the power contained in the brackets following, D is the pulmonary diffusing capacity for CO in ml. STPD/mm. Hg × sec, V_A is the total alveolar gas volume during the period of breathholding in ml. STPD, and Pb is the barometric pressure in mm. Hg minus 47 mm. Hg.

In order to derive Equation *1*, Krogh had to assume that (a) the CO concentration was equal in all alveoli of the lungs and (b) that the plasma CO tension was negligible. The calculations of Rauwerda (3) suggest that it is permissible to assume that little or no CO gradient would exist within the gas of a single alveolus. However, recent studies on uneven ventilation (4) make it highly probable that the initial alveolar CO concentration varies among different alveoli after a single inspiration of a gas containing CO. Assumption (b) has been questioned by Roughton (5) because the rate of combination of CO with hemoglobin in human red cells at 37° C. is sufficiently slow in relation to the rate of CO diffusion from alveolar gas into the capillary blood that a significant tension of CO must be present in the blood. At this juncture it should be pointed out that capillary CO tension may be significant either because the mean capillary COHb concentration is large or because the diffusing capacity in the alveolus is large in relation to the rate of combination of CO and Hb. This latter CO tension will disappear in the arterial blood, because the reaction of CO and Hb will continue after the blood has left the alveolus.

Therefore, the two assumptions (a) and (b) are invalid to some degree. In a separate paper

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(6) the uptake of CO from a single alveolus during breathholding and during steady state breathing of a gas mixture containing CO is investigated. Obviously the entire discussion cannot be reproduced here. For the purpose of this paper the decrease in alveolar CO concentration with time during breathholding can be expressed as

$$F_A = F_{A_0} \exp\left(-\frac{DPbCt}{V_A}\right), \qquad (2)$$

where C is a correction factor[4] to allow for the fact that the plasma CO tension is not negligible.

Since it can no longer be assumed that the alveolar CO concentration is equal throughout the lung, the changes of CO concentration in the gas that we can sample, namely the mixed[5] expired alveolar gas, must be studied. The mixed expired alveolar CO concentration can be described as the sum of the concentrations of the different alveoli, weighted according to their fractional contribution to the expired sample. Thus:

$$\overline{F_{AE}} = \sum_1^N \phi_i F_{AEi}, \qquad (3)$$

where $\overline{F_{AE}}$ is the mixed expired alveolar CO concentration, ϕ_i is the fraction contributed by the ith alveolus, F_{AE_i}[6] equals the CO concentration in the ith alveolus, and N is the number of alveoli. When the alveolar CO concentration as given by Equation *2* is substituted in Equation *3*, we obtain

$$\overline{F_{AE}} = \sum_1^N \phi_i F_{AOi} \exp\left(-\frac{D_i PbC_i t}{V_{Ai}}\right). \qquad (4)$$

F_{AOi}, the initial CO concentration in the ith alveolus equals $\frac{(V_T - V_D)_i}{V_{Ai}} F_I$, where $(V_T - V_D)_i$ equals the inspired volume reaching the alveolus, V_{Ai} equals the alveolar volume during the period of breathholding, and F_I equals the inspired CO concentration (dry). Substituting this expression for F_{AOi} in Equation *4*, we obtain

$$\overline{F_{AE}} = F_I \sum_1^N \phi_i \frac{(V_T - V_D)_i}{V_{Ai}} \exp\left(-\frac{D_i PbC_i t}{V_{Ai}}\right). \qquad (5)$$

$\overline{F_{AE}}$ consists of the sum of a series of exponential decay terms, each exponent of which may be different, and equals $-\frac{D_i PbC_i}{V_{Ai}}$. The zero intercept or the value of $\overline{F_{AE}}$ when $t = 0$, is $F_I \sum_1^N \phi_i \frac{(V_T - V_D)_i}{V_{Ai}}$. This intercept represents the sum of the initial concentration in each alveolus multiplied by the fraction of the expired sample contributed by that alveolus. Since there are a great number of alveoli in the lung one would expect to find many with the same decay constant. Such similar alveoli can be considered together and will be termed a *diffusing phase*. The different alveoli constituting a "phase" are not necessarily located near each other.

It is interesting to consider the $\log \overline{F_{AE}}$, because this form is useful in solving for D, the pulmonary diffusing capacity.

$$\log \overline{F_{AE}} = \log F_I + \log \sum_1^N \phi_i \frac{(V_T - V_D)_i}{V_{Ai}} \times \exp\left(-\frac{D_i PbC_i t}{V_{Ai}}\right). \qquad (6)$$

This need not be a straight line, as is suggested by consideration of Equations *1* or *2* alone. However, if all exponents are equal, then Equation *6* becomes

$$\log \overline{F_{AE}} = \log F_I \sum_1^N \phi_i \frac{(V_T - V_D)_i}{V_{Ai}} - \frac{D_i PbC_i t}{V_{Ai}} \log_{10} e. \qquad (7)$$

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[4] $C = \dfrac{1}{1 + \dfrac{D}{\theta V_c}}$.

θ is the average rate of combination of CO with the hemoglobin in the corpuscle under specific conditions of oxygenation, temperature and pH encountered along the pulmonary capillary, expressed in ml. CO STPD combining/sec. × ml. of blood × mm. CO tension plasma. V_c is the volume of blood in the pulmonary capillaries in ml.

[5] "Mixed" is indicated by a bar over the symbol ($\overline{F_{AE}}$ which equals *mixed* expired alveolar CO concentration), and signifies that the sample contains gas from more than one alveolus. "Mean" is indicated by a heavy bar over the symbol ($\overline{F_{AE}}$ which equals *mean* expired alveolar CO concentration) and signifies that the sample contains gas collected during an entire respiratory cycle.

[6] The CO concentration is considered equal throughout an alveolus and the time of expiration is considered negligible so that the expired alveolar concentration equals the alveolar concentration.

This is a straight line relationship. Thus one of the conditions for log $\overline{F_{AE}}$ versus time to be a straight line as Krogh assumed is that the decay constants of all parts of the lung must be equal. It is important to note that if the exponents are all equal log $\overline{F_{AE}}$ will be linear regardless of the unevenness of ventilation. The exponents will be equal if $\frac{D_iC_i}{V_{A_i}}$ is equal in the different alveoli. C_i $\left(\text{which equals } \frac{1}{1+\frac{D_i}{\theta_i V_{ci}}}\right)$ depends on the diffusing capacity of the alveolus (D_i) the volume of capillary blood in the alveolus (V_{ci}) and on the rate of combination of CO with Hb (θ_i). Although θ varies with alveolar O_2 tension, increasing slightly with a decreasing O_2 tension, the factor C_i would be expected to vary less throughout the lung than D_i/V_{A_i}, the diffusing capacity per unit alveolar gas volume. The extent of variation of these quantities in the lung is unknown at present, although the disappearance of CO from the alveoli is not exponential (1). The problem of obtaining the true total pulmonary diffusing capacity in the face of variations in D_i/V_{A_i} throughout the lung is a matter for future study.

B. *The Uptake of CO under "Steady State" Conditions*

Under special conditions and for short periods of time, a subject who has started to breathe CO reaches a so-called "steady" state. In this condition, $\overline{F_A}$, the mean alveolar CO concentration in a single phase is constant from breath to breath. In other words, the amount of CO removed from the inspired gas during a respiratory cycle equals that being taken up by the blood. Stated mathematically when venous COHb equals 0,

$$f(V_T - V_D)(F_I - \overline{F_{AE}}) = D\overline{F_A}PbC, \quad (8)$$

where F_I is the inspired concentration of CO (dry), f is the frequency of respiration per second, $\overline{F_{AE}}$ is the mean in time of the expired alveolar CO concentration, and $\overline{F_A}$ is the mean in time of the alveolar concentration. $\overline{F_{AE}}$ does not necessarily equal $\overline{F_A}$, because $\overline{F_A}$ is a mean over the whole respiratory cycle, while $\overline{F_{AE}}$ is expelled only during the expiratory phase of the respiratory cycle. However, it is more convenient to let $\overline{F_A} = \overline{F_{AE}}$ in Equation *8* and change D to D′. This indicates that the value of D′ obtained from the application of the "steady state" principle is not necessarily equal to the true D. If $\overline{F_{AE}}$ is substituted for $\overline{F_A}$ in Equation *8* and the latter is then solved for $\overline{F_{AE}}$, we obtain

$$\overline{F_{AE}} = \frac{F_I}{1+\frac{D'PbC}{f(V_T - V_D)}}. \quad (9)$$

However, just as in the case of the fall in CO during breathholding, we cannot obtain gas from the individual alveoli, but must be content with an average sample from the whole lung. For the "steady state" circumstances this expired sample is a mean mixed expired sample ($\overline{\overline{F_{AE}}}$): it is a mean in time over most of the respiratory cycle and a mixture of contributions from different alveoli.

$$\overline{\overline{F_{AE}}} = \sum_1^N \frac{(V_T - V_D)_i}{V_T - V_D}\overline{F_{AE_i}}. \quad (10)$$

Substituting the value of F_{AE} for an individual alveolus given by Equation *9* in Equation *10*, we obtain

$$\overline{\overline{F_{AE}}} = \frac{F_I}{V_T - V_D}\sum_1^N \frac{(V_T - V_D)_i}{1+\frac{D_i'PbC_i}{f(V_T - V_D)_i}}. \quad (11)$$

The total pulmonary diffusing capacity $\left(\sum_1^N D_i' = D'\right)$ has been computed by dividing the CO uptake per minute by the mean mixed expired alveolar CO tension (2, 5). It is important to note that this is not correct unless the lung is considered a single diffusing/ventilating phase. Thus if total pulmonary diffusing capacity is calculated by dividing the total CO uptake per minute by the mean mixed expired alveolar CO tension,[7]

$$aD' = \frac{f(V_T - V_D)(F_I - \overline{\overline{F_{AE}}})}{Pb\overline{\overline{F_{AE}}}}. \quad (12)$$

[7] This, of course, assumes the plasma CO tension is negligible, *i.e.*, C = unity. Obviously this is not true, but (a) it has been done (b) it has a certain clinical usefulness and (c) it does not alter the force of the discussion. Any alternate presentation appears unnecessarily complicated.

aD' is the apparent pulmonary diffusing capacity of the whole lung. This letter "a" for "apparent" will be used to indicate that the value of D' was obtained from mixed alveolar air from the entire lung without regard for variations in the diffusing-ventilating ratios of the different alveoli. When the value of $\overline{F_{AE}}$ from Equation *11* is substituted herein

$$aD' = \frac{f(V_T - V_D)}{Pb}\left[\frac{(V_T - V_D)}{\sum_1^N \frac{(V_T - V_D)_i}{1 + \frac{D_i'PbC_i}{f(V_T - V_D)_i}}} - 1\right]. \quad (13)$$

aD' will equal the true total D' if $\frac{D_iPbC_i}{f(V_T - V_D)_i}$ is the same throughout the lung. As discussed above, this condition is dependent mainly on $D_i'/(V_T - V_D)_i$ remaining the same throughout since C_i has been considered unity. The condition of constant $D_i'/(V_T - V_D)_i$ is actually a condition of maximum efficiency, where the ventilatory gas is distributed proportionally to the available diffusing surface. Any deviation from this ideal condition will make aD' less than the actual D'. It must be noted that this condition is not the same as the major requirement that mean expired CO fall exponentially with time, which is that $\frac{D_i}{V_{A_i}}$ be constant throughout the lung.

The fractional CO uptake

$$\left(\frac{\text{CO inspired in ml./min.}}{\text{CO absorbed in ml./min.}}\right)$$

has been investigated by a number of workers (7–9). The CO uptake per minute of any alveolus equals $f(V_T - V_D)_i(F_I - \overline{F_{AE}})_i$ (see Equation *8*). Therefore the CO uptake per minute for the whole lung is $f\sum_1^N (V_T - V_D)_i(F_I - \overline{F_{AE_i}})$. Using the value of $\overline{F_{AE_i}}$ given by Equation *9*, since the inspired CO is fV_TF_I, fractional CO uptake in per cent

$$= \frac{100}{fV_T}\sum_1^N \frac{1}{\frac{1}{f(V_T - V_D)_i} + \frac{1}{D_i'PbC_i}}. \quad (14)$$

It is clear from this equation that changes in fractional CO uptake cannot be related to changes in pulmonary diffusing capacity unless the relationship of D', $V_T - V_D$ and C are known for all parts of the lung. Disregarding for the moment any question of variations in $D_i'C_i/(V_T - V_D)_i$ throughout the lung, an increase in D' will cause an increased fractional CO uptake which seems reasonable. Note however that an increase in minute ventilation (fV_T) will cause a decrease in fractional CO uptake (9) (except at extremely low tidal volumes). If we include the possibility of variation in $D_i'C_i/(V_T - V_D)_i$ in the lung, a change in ventilation pattern could cause a decrease in fractional CO uptake even though total diffusing capacity and total ventilation were kept constant. It can be shown that the fractional CO uptake will be maximal when the two terms of the denominator of Equation *14* are in the same ratio in all phases. The fractional CO uptake in the presence of uneven distribution of gas within the lung will be influenced more by the diffusing capacity of overventilated parts of the lung than of the underventilated parts.

It must be emphasized that the assumption that respiration is a continuous process is certainly not true and use of Equation *14* to correct for the effect of changes in effective ventilation $f(V_T - V_D)$ upon the fractional CO uptake may fail in extreme cases for this reason.

C. *The Effect of the Presence of COHb in the Mixed Venous Blood*

In all discussions up until this point, it has been assumed that the mixed venous blood had no significant amount of COHb in it. Under normal circumstances this is true, but after periods of breathholding approaching a minute, the alveolar CO concentration falls to levels where the tension of CO in equilibrium with the venous blood becomes important in smokers. Therefore, it is often necessary to make a correction for the mixed venous COHb. This problem is discussed in more detail in another paper (6) but for present purposes it will suffice to say that since all the processes discussed above are exponential, we should deal with the difference between the alveolar CO concentration and its value at equilibrium, rather than the alveolar CO concentration alone.

In the cases already discussed the equilibrium

value has been zero, but in cases where the mixed venous COHb is significant, the equilibrium value becomes greater than zero. When the alveolar O_2 tension is high enough to ensure saturation of the Hb, as when 100 per cent O_2 is inspired, the equilibrium CO concentration can be calculated from the Haldane relationship (10),

$$F_c = \frac{F_{cO_2}COHb}{MO_2Hb}. \qquad (15)$$

F_c is the CO concentration, F_{cO_2} the O_2 concentration of gas in equilibrium with the blood. COHb and O_2Hb are the concentrations of the respective compounds expressed in volumes of gas (STPD) combined per volume of blood. Aside from questions as to the exact value of M, which is approximately 210, this equation is almost precisely correct when 100 per cent O_2 is inspired, because the capillary O_2 tension becomes equal to the alveolar O_2 tension near the venous end of the capillary, and the CO tension in the plasma will build up from what it was in the mixed venous blood to correspond to the value given by Equation *15* equally rapidly. With lower alveolar O_2 tensions the use of the Haldane relation becomes less exact, but because the COHb concentration is almost always less than 0.02 ml. gas per ml. blood (2 vols. per cent) in the mixed venous blood, the error involved is usually negligible.

Substituting (alveolar CO concentration-equilibrium alveolar CO concentration) for alveolar CO concentration, the expired alveolar CO concentration of a single alveolus during breathholding following a single inspiration of a gas containing CO becomes

$$F_{AE} = \left[F_I \frac{V_T - V_D}{V_A} - \frac{F_{cO_2}COHb}{MO_2Hb}\right] \times \exp\left(-\frac{DPbCt}{V_A}\right) + \frac{F_{cO_2}COHb}{MO_2Hb}. \qquad (16)$$

Similarly, the mixed expired alveolar CO concentration during breathholding (Equation *5*) becomes

$$\overline{F_{AE}} = \sum_1^N \phi_i \left[\frac{(V_T - V_D)_i F_I}{V_{Ai}} - \frac{COHbF_{cO_2i}}{MO_2Hb}\right] \times \exp\left[-\frac{D_i PbC_i t}{V_{Ai}}\right] + \sum_1^N \phi_i \frac{COHbF_{cO_2i}}{MO_2Hb_i}. \qquad (17)$$

The last term containing COHb is a summation, weighted according to the contribution of each phase. In practice the entire term is small, and can be calculated from average values of COHb, O_2Hb and F_{cO_2}.

Analogous equations can be obtained for the steady state conditions, but are more involved than the equations above and will not be reproduced here. It is more convenient to change the variable in the equations already given in the previous section on the steady state conditions. This simply means subtracting the equilibrium value of CO concentration ($COHbF_{cO_2}$)/MO_2Hb from F_A and F_I, and substituting these new values in the given equations.

D. *The Relation between Expired Alveolar CO Concentration and Time Considering Respiration a Continuous Process and the Lung as a Single Bag*

The circumstances considered in this section deal with a subject who starts to breath a mixture containing CO. The equations are concerned with the washout of the original gases in the alveoli, and the attainment of a steady state where the CO brought to the alveoli by the ventilation equals that carried away by the blood. This washout process is essentially complete before the mixed venous blood COHb becomes significant. Respiration is considered to be a continuous process. Variations in ventilation, or diffusion throughout the lung are ignored, because of complexity, and the lung is considered a perfectly mixed bag.

The rate of change of CO contained in the lung gases $\frac{(d\overline{F_A}V_A)}{dt}$ equals the net rate at which CO is carried to the lung by ventilation $(V_T - V_D)f(F_I - \overline{F_{EA}})$ less the rate at which it is carried away by diffusion into the blood $(\overline{F_A}aD'PbC)$. Thus

$$\frac{d\overline{F_A}V_A}{dt} = f(V_T - V_D)(F_I - \overline{F_{AE}}) - \overline{F_A}aD'PbC. \qquad (18)$$

The difference between the inspired and expired gas volumes has been ignored. Note that the factor C has been included to allow for the fact that plasma CO tension is not always negligible. Since respiration has been considered a continuous process, mean expired alveolar CO ($\overline{F_{AE}}$) and

mean alveolar CO ($\overline{F_A}$) are implicitly considered equal, and therefore aD′ is used rather than aD as discussed earlier. Furthermore, since all unevenness of ventilation and diffusion throughout the lung is ignored, aD′ is employed rather than D′. The solution of Equation *18* is

$$\overline{F_{AE}} = \frac{F_I}{1 + \frac{aD'PbC}{f(V_T - V_D)}}\left[1 - \exp\left(-\frac{t}{V_A} \times \left[f(V_T - V_D) + aD'PbC\right]\right)\right]. \quad (19)^8$$

E. *The Uptake of CO under "Steady State" Conditions over Long Time Periods*

If the circulation time of the blood is negligible in comparison with the period of time under consideration, then the body blood volume (B), which includes myoglobin, may be assumed mixed at any instant, and the washout time of the gas in the lungs can be neglected. If in addition, ventilation-perfusion and ventilation-diffusion ratios are assumed equal throughout the lung and ventilation is assumed a continuous process, then the change in total COHb content of the blood at any instant will equal the amount of CO diffusing across the pulmonary membrane. Stated symbolically,

$$\frac{dCOHb}{dt} B = \left[\overline{F_A} - \frac{COHbF_{cO_2}}{MO_2Hb}\right] aD'PbC. \quad (20)$$

The right hand term can be looked on as the pressure difference between the CO in the alveolar gas and that in the blood, multiplied by the pulmonary diffusing capacity, corrected for the slowness of the reaction of CO with Hb by the factor C. aD′ is used because the expired alveolar CO concentration is considered equal to the alveolar CO concentration in a continuous process, demanding a prime. Variations in diffusing capacity and ventilation throughout the lung have been ignored, demanding the "a." Because ventilation is continuous, the CO increase in the blood must equal the CO lost in the ventilatory exchange, or

$$\frac{dCOHb}{dt} B = f(V_T - V_D)(F_I - \overline{F_A}). \quad (21)$$

[8] Dr. Philip Hugh-Jones has told us that an equation similar in many respects to this one has been developed at the M.R.C. Pneumoconioses Unit in Wales, and will be reported in the future.

Equations *20* and *21* are combined and solved for $\overline{F_A}$ in terms of COHb. This value of $\overline{F_A}$ is then inserted in Equation *20* and the resulting differential form solved. The result is

$$COHb = \frac{F_I S}{F_I + \frac{F_{cO_2}}{M}} \times (1 - \exp(-xt + yCOHb)), \quad (22)$$

where

$$x = \frac{[aD'PbC + f(V_T - V_D)]\left(F_I + \frac{F_{cO_2}}{M}\right)^2}{BaD'PbCf(V_T - V_D)\left(\frac{F_{cO_2}}{M}\right)S},$$

$$y = \frac{\left(F_I + \frac{F_{cO_2}}{M}\right)}{S\frac{F_{cO_2}}{M}}$$

and S is the capacity of the blood for CO or O_2 in ml. gas (STPD) per ml. blood. Although this relationship is complicated it will describe satisfactorily the published data of Forbes, Sargent, and Roughton (7) and Pace, Consolazio, White, and Behnke (8). The equations of these investigators (7, 8) and of Hatch (11) can be shown to be approximate forms of Equation *22*. Even this complicated equation contains a number of simplifying assumptions, which are listed above, plus an additional assumption that C does not vary significantly during an experiment, which is probably true enough, provided the arterial blood is not unsaturated. This equation is useful, either to obtain estimates of aD′ or knowing aD′ and C, to predict the rate of build up of COHb in the blood. Variations in C are primarily a function of changes in the alveolar O_2 tension.

SUMMARY

Preliminary investigations on the Krogh CO method of measuring the diffusing capacity of the lung revealed the necessity for re-examining the theory of CO uptake from the lungs. Equations have been derived which describe mixed expired alveolar CO concentration, during breath-holding following a single inspiration of a gas containing CO and during "steady state" breathing of a mixture containing CO, when the alveoli have different ventilation rates, alveolar gas vol-

umes, and diffusing capacities. The effect of significant amounts of COHb in the mixed venous blood is discussed. Two equations are also derived describing the expired alveolar CO concentration during the breathing of a mixture containing CO. The first deals with the initial "washout" of the lung gases, and the attainment of the gas "steady state." The second deals with the much slower build-up of COHb in the mixed venous blood.

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THE ABSORPTION OF CARBON MONOXIDE BY THE LUNGS DURING BREATHHOLDING [1]

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In 1915, M. Krogh reported the use of carbon monoxide in an ingenious technique for the measurement of the pulmonary diffusing capacity in man (1). In her method a maximal inspiration of a gas mixture containing CO was made from residual volume and followed immediately by an expiration of at least one liter of gas. The breath was held at the remaining volume for 6 to 10 seconds, and then a maximal expiration was made. The terminal volumes of the two expirations were considered to be alveolar gas and were analyzed for CO concentration. M. Krogh assumed the CO concentration decayed exponentially and derived the following equation to describe this decay.

$$F_A = F_{A_0} \exp\left(-\frac{DPbt}{V_A}\right) \qquad (1)$$

F_A is alveolar concentration of CO (dry) at time t (Krogh's final sample); F_{A_0} is the alveolar concentration (dry) at time zero (Krogh's initial sample); "exp" is e, the base of the natural logarithms, raised to the power contained in the brackets following; D is the pulmonary diffusing capacity for CO in ml. STPD/mm. Hg × sec.; V_A is the total alveolar gas volume in ml. STPD during the period of breathholding, obtained from a spirometer tracing and from measurement of functional residual capacity; t is time in seconds between the delivery of the two gas samples; and Pb is the total barometric pressure minus 47 mm. Hg. The pulmonary diffusing capacity (D) was calculated by substituting the values obtained for F_A, F_{A_0}, t, Pb and V_A in a rearrangement of Equation *1*.

In order to derive Equation *1*, Krogh had to make the following major assumptions: (a) that the CO concentration in the first sample was representative of all alveolar gas; and (b) that the CO tension in the pulmonary capillary[5] plasma (P_c) was negligible. Although her technique involves delivering two expired alveolar samples, these are actually parts of the same expiration, being separated by the breathholding period. It is now known that these two alveolar samples would not have the same initial CO concentration (2). Furthermore, Roughton has indicated that P_c may not be negligible (3), casting doubt on the validity of the second assumption. Therefore, it was decided to reinvestigate the disappearance of CO from the human lung during breathholding following a single inspiration of a gas mixture containing CO. Recently developed physical methods of gas analysis, in particular mass spectrometry and infrared absorption techniques, were used throughout.

At first Krogh's experiments were repeated as she described them (1) and values for the pulmonary diffusing capacity were obtained which agreed with those she reported. However, the calculated value of the pulmonary diffusing capacity varied with the length of time the breath was held, which is incompatible with Equation *1*. In order to investigate this phenomenon further, the experimental procedure was modified. Only one expired sample was obtained, and the initial CO concentration (F_{A_0}) was computed from the dilution of an insoluble gas (He) contained in the inspired mixture. This obviated the necessity of comparing CO concentration in two different alveolar gas samples, and thereby eliminated the need for Krogh's first premise above. The breath was held for different lengths of time, and when the resulting expired alveolar CO concentrations

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[5] The term "pulmonary capillary" refers to that part of the pulmonary vascular bed which is exposed to alveolar gas.

were suitably corrected for variations in the dilution of the inspired mixture, a plot of log F_A against time was obtained. This plot of log F_A against the duration of breathholding was not a straight line in the seven subjects studied, as Krogh had assumed. Some pertinent theoretical considerations of these results are presented in the accompanying paper (4).

METHODS

The concentration of CO was measured in an infrared gas analyzer.[6] It had an analysis chamber of 60 ml. capacity which required a 200 ml. sample to flush it completely. The response of the instrument to a sudden change in CO concentration was 90 per cent complete in 0.4 second. Background noise was equivalent to from 0.0005 to 0.001 per cent CO. The calibration curve was alinear, so that the proportional precision varied with the CO concentration. Representative limits of overall error were ±0.005 per cent at 0.400 per cent CO and ±0.001 per cent at 0.010 per cent CO. The instrument was also sensitive to water vapor and carbon dioxide. Forty-seven mm. Hg of water vapor gave a response equivalent to 0.002 per cent CO; this increased to 0.010 per cent CO over a matter of months, as the detector unit aged. This response was additive to the CO deflection, so in practice either a zero correction was made for water vapor in the breath or the samples were dried with $CaCl_2$ before analysis. Five per cent CO_2 gave a response equivalent to 0.001 to 0.002 per cent CO. In experiments in which this constituted a significant error, a suitable correction was made.

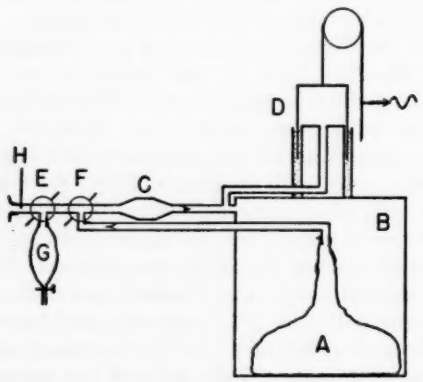

FIGURE 1

Arrangement of apparatus. (A) is a 300 litre plastic bag, (B) a 300 litre metal box, (C) a capacitance manometer resistance flow meter (Lilly), (D) a 6 litre spirometer, (E) and (F) are 2 cm. bore three way metal taps, (G) a 6 litre rubber sample bag, and (H) the inlet to the mass spectrometer sampling system. The circuit is fitted with unidirectional flow valves.

[6] Liston-Becker Instrument Co., Stamford, Conn.

A CO mixture of known concentration was used to calibrate the infra red gas analyzer for each analysis.

Helium, nitrogen, oxygen, and carbon dioxide were analyzed with a single channel, continuously sampling mass spectrometer.[7] This instrument was precise to at least 1 per cent of full scale. The gas sample was drawn continuously into the spectrometer at a rate of 0.3 ml. per second. The 90 per cent response time was 0.12 second, of which 0.06 second represented the time the gas sample took to flow through the inlet system to the spectrometer. Since this inlet delay was constant, the useful 90 per cent response time was 0.06 second. The instrument was sensitive to 0.05 per cent N_2, 0.25 per cent He, 0.05 per cent O_2 and 0.04 per cent CO_2, and gave a linear output.

Respiratory flow rates were recorded with a capacitance manometer flowmeter (Lilly). The mass spectrometer and flowmeter outputs were recorded simultaneously by means of a double channel D.C. amplifier and pen motor[8] on a paper record running at either 5 or 25 mm. per second. Gas volumes were measured in either a 6 or 100 liter spirometer.

The diagram of the apparatus used is shown in Figure 1. Inspiration was made from a neoprene bag (A) contained in a sealed metal chamber (B). Expiration was made through the flowmeter (C) into the chamber (B). A 6 liter spirometer (D) recorded changes of total volume in the system. Two 2 cm. diameter three way taps (E and F) were provided. Tap E enabled the subject to breathe out the first liter of an expiration into the circuit, and to collect the remainder in a rubber bag (G) close to the mouthpiece. Tap F permitted closure of the inspiratory bag to prevent loss of the inspired gas. The inlet (H) to the mass spectrometer sampling tube was at the mouthpiece.

In an actual experiment the tubing from bag A to tap F was first flushed with the inspired gas, and the sampling bag evacuated and closed off. The inspired gas mixture was usually 0.3 per cent CO, 10 per cent He, 20 per cent O_2 and 70 per cent N_2. The seated subject, wearing a nose clip, stopped breathing at the end of a normal expiration, put the mouthpiece in his mouth and expired to his residual volume. He then rapidly inspired the desired volume of the gas mixture containing CO, held his breath for a predetermined time, and rapidly expired to residual volume, turning tap E to collect the sample after a liter or more of gas had been expired. Expiratory flow rate and He concentration were recorded simultaneously during this procedure. This discarded gas volume was considered sufficient to wash out the respiratory dead space, so that the collected sample was "alveolar gas." This sample was then analyzed for CO. Its average He concentration as a fraction of that inspired could be obtained from the records taken during the experiment. The time of breathholding was measured from the start of expiration of the sample, as indicated by the flowmeter. This experiment was repeated with the breath held for different periods of time such as 60, 10, 50, 20, 40, 30 seconds in that order,

[7] Consolidated Engineering Corp., Pasadena, California.

[8] Brush Development Co., Cleveland, Ohio.

so that the relation between mixed expired alveolar CO concentration and time could be plotted.

Helium was added to the inspired gas mixture to provide a method of obtaining the alveolar CO concentration at time zero without collecting two gas samples as Krogh (1) did. The mixed expired alveolar CO concentration at time zero in the collected sample (F_{AEO})[9] was computed from the data by assuming that He was insoluble in blood and tissue, and that CO was not absorbed by blood or tissue to any significant extent until the inspired and residual gases were mixed. The validity of these assumptions is discussed later. The mixed expired alveolar concentration of CO at time zero could therefore be calculated as follows:

$$\overline{F_{AEO}} = \frac{\overline{F_{AEHe}}}{F_{IHe}} \times F_I \qquad (2)$$

Where: $\overline{F_{AEO}}$ = Calculated dry CO concentration of the collected sample at time zero.[10]

$\overline{F_{AEHe}}$ = Mixed expired alveolar He concentration dry.

F_I = Inspired CO concentration dry.

F_{IHe} = Inspired He concentration dry.

The original alveolar concentration of CO calculated as described above varied a great deal because of differing inspired concentrations of CO and differing relations between inspired and residual gas volumes, in spite of conscious effort to maintain them constant. For convenience in plotting, all values of $\overline{F_{AEO}}$ were adjusted to an initial alveolar concentration of 0.2 per cent CO dry. That is, any value of $\overline{F_{AE}}$ was multiplied by the fraction $\frac{0.2 \text{ per cent CO}}{\overline{F_{AEO}}}$ before it was plotted. The justification for this operation is given in Equation 5 of Reference 4.

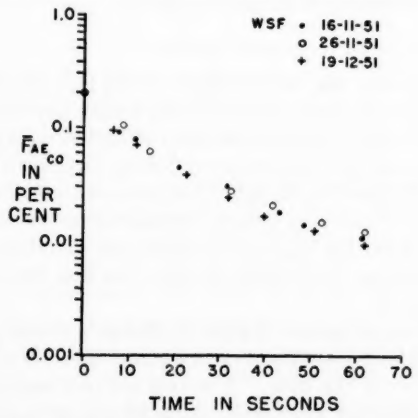

FIG. 2. REPEATED CO DISAPPEARANCE CURVES IN THE SAME SEATED SUBJECT ON DIFFERENT DAYS

Inspired gas mixture contained 20% O_2. Results typical of those on seven normal men.

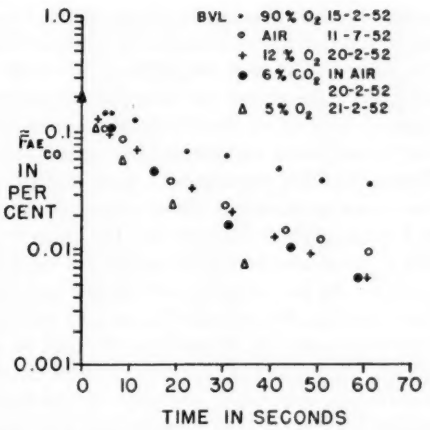

FIG. 3. THE EFFECT OF ALTERING THE O_2 AND CO_2 TENSIONS IN THE INSPIRED GAS MIXTURES UPON THE CO DISAPPEARANCE CURVE IN THE SEATED SUBJECT

Results typical of four normal men.

Assuming an alveolar gas volume of 4,000 ml., a pulmonary blood flow of 6,000 ml. per minute, a volume of watery tissue exposed to the alveolar gas of 1,000 ml. (5), and a partition coefficient for He at 37° of 0.0098 (6), the He dissolved in the blood and tissues in a minute would be less than $\frac{(1,000 + 6,000)}{4,000} \times .0098$ or approximately 2 per cent of the He in the alveolar gas. For the purposes of the present experiments this error can be neglected, and the He can be assumed insoluble in blood and tissue.

RESULTS

Figure 2 shows three complete series of experiments on one subject on different days inspiring a CO-He mixture in air. It is clear that CO does not disappear exponentially from the alveoli during breathholding. The curvature is typical of 28 separate series of experiments on seven seated normal men 29 to 37 years of age.

The effect of varying the alveolar pO_2 was also tested because present information on the kinetics of the CO + Hb reaction predicts that lowering the alveolar O_2 tension would increase the rapidity of disappearance of CO from the lung (7). Roughton (3) has suggested that a significant capillary CO tension exists when breathing 20 per cent O_2, but that the true pulmonary diffusing capacity might be obtained in spite of this by observing the diffusing capacity at de-

[9] This terminology is discussed in the previous paper (4).

[10] The duration of delivery of the collected sample will be ignored at this point.

creasing alveolar O_2 concentrations and extrapolating to a point where it might be expected that the capillary pCO was negligible. In order to investigate these points the effect of varying the alveolar O_2 tension on the CO uptake was studied in four normal men and typical results are shown in Figure 3. The experiments were carried out in the same manner as those described above except that, after breathing air, the subject inspired a gas mixture containing 0.3 per cent CO plus either 90 per cent O_2 and 10 per cent He; 12 per cent O_2, 78 per cent N_2 and 10 per cent He; or 5 per cent O_2, 85 per cent N_2 and 10 per cent He. As predicted (7) when alveolar oxygen concentration decreased, the rate of CO disappearance increased. This effect did not reach an obvious maximum with decreasing alveolar O_2 tensions nor was it possible to extrapolate the data to lower alveolar O_2 tensions. There was a significant difference in the CO uptake at different alveolar O_2 tensions even with breathholding for only 3 to 5 seconds.

The effect of adding 6 per cent CO_2 to the inspired mixture (6 per cent CO_2; 19 per cent O_2; 10 per cent He; approximately 0.3 per cent CO, 65 per cent N_2) is also presented in Figure 3. There is a tendency for the alveolar CO concentration after about 20 seconds to be lower when 6 per cent CO_2 is added to a given inspired mixture. This was typical of the four subjects studied.

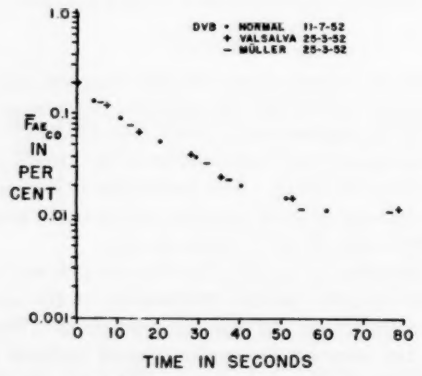

FIG. 4. THE EFFECT OF ALTERING THE INTRAPULMONIC PRESSURE DURING BREATHHOLDING ON THE CO DISAPPEARANCE CURVES IN THE SEATED SUBJECT

Intrapulmonic pressure approximately +40 mm. Hg during Valsalva; −40 mm. Hg during Müller. Results typical of two normal subjects.

Since the intrapulmonic pressure during breathholding may vary considerably, and any changes in it might affect the shape of the CO disappearance curve, the effects of extremes of intrapulmonic pressure were investigated. CO disappearance curves were obtained in two normal males, the subject maintaining voluntary positive (Valsalva) or negative (Müller) intrapulmonary pressure with the glottis closed, during the period of breathholding. The pressures so obtained were estimated to be roughly plus and minus 40 mm. Hg, respectively. These maneuvers require a high degree of cooperation, but in the trained experimenters used, it seemed reasonable to assume that the intrapulmonary pressure varied more between these two states than it would during a single experiment under the usual breathholding conditions. A typical set of disappearance curves is shown in Figure 4 along with a normal curve, the data of which are given in Table I. There is no significant difference between the data.

Since M. Krogh found that the diffusing capacity for CO varied with lung volume, the CO disappearance curve was investigated at different total lung volumes. We did not find a significant increase in pulmonary diffusing capacity with increasing lung volume. These results will form the substance of a future report.

DISCUSSION

Because the initial slope of log $\overline{F_{AE}}$ plotted against time may be two to more than five times greater than the slope between 40 and 60 seconds, and since the pulmonary diffusing capacity (D) as calculated by Krogh (1) is proportional to the slope, D can vary greatly depending on the portion of the log $\overline{F_{AE}}$ curve used in the calculation. The longer the breath is held, the less the apparent D.

Another source of error in Krogh's technique arises because of the presence of non-uniform ventilation of the lung. The two alveolar samples she collected were really parts of one expiration interrupted by a period of breathholding. It is known that the first part of any expiration (corresponding to Krogh's initial sample) contains higher concentrations of the gases that were inspired in the preceding inspiration than the later parts of the expiration (Krogh's final sample) (2). Thus Krogh's early gas sample

TABLE I*

Exp.	a Time *secs.*	b Vol. of inspired gas *L. STPD*	c $\overline{F_{AE_{O_2}}}$ % *dry*	d He dilution ratio	e $\overline{F_{AE_O}}$ (k × d) % *dry*	f $\overline{F_{AE}}$ % *dry*	g $\overline{F_{AE}}$ adjusted to 0.2% initially $\left(F \times \frac{.2}{e}\right)$ % *dry*	h F_c % *dry*	i $\overline{F_{AE}}$ corr. for blood COHb (f–h) % *dry*	j $\overline{F_{AE}}$ corr. for blood COHb and adjusted to 0.2% initially $\left(i \times \frac{.2}{e}\right)$ % *dry*
1	60.6	3.82	15.0	0.80	0.264	0.014	0.011	0.0010	0.013	0.010
2	4.6	3.70	20.3	0.75	0.247	0.146	0.120	0.0017	0.144	0.117
3	51.2	3.40	16.5	0.82	0.270	0.022	0.016	0.0025	0.020	0.015
4	10.6	3.73	18.6	0.76	0.250	0.108	0.088	0.0032	0.105	0.085
5	40.2	3.84	16.4	0.77	0.254	0.028	0.022	0.0040	0.024	0.019
6	20.6	3.72	18.2	0.82	0.270	0.068	0.051	0.0048	0.063	0.049
7	29.0	3.55	17.3	0.76	0.250	0.046	0.037	0.0055	0.040	0.033

* Inspired gas contained 0.327 per cent CO (k), 22.6 per cent O_2, about 10 per cent He and the remainder N_2, all expressed as dry gas.

Column (a) is explained elsewhere in the text. (b) is self-explanatory. (c) $F_{AE_{O_2}}$ is the mixed expired O_2 concentration in the alveolar sample (dry). (d) The He dilution ratio equals mixed He concentration (dry) of the collected expired alveolar sample/inspired He concentration (dry), both in scale deflection as measured at the mouthpiece. (e) $\overline{F_{AE_O}}$ equals the CO concentration that was in the sample initially and is computed by multiplying the inspired CO concentration (0.327 per cent) times the He dilution ratio (d). (f) Collected sample analysis in per cent CO (dry). This has been corrected for an analytical error of +0.0015 per cent CO because of presence of CO_2. (g) The sample analysis adjusted to 0.2 per cent CO (dry) initially. (h) F_c (per cent dry) is the "average" concentration of CO in the gas in equilibrium with blood in the pulmonary capillary during each experiment. The mixed venous COHb concentration was estimated immediately before and after the series of experiments using Siösteen and Sjöstrand's technique (13). Using the Haldane relationship and assuming a mean alveolar O_2 concentration of 17.4 per cent (dry) during an experiment, these values were initially 0.0013 ml. CO/ml. blood: finally 0.0134 ml. CO/ml. blood. The concentration of CO in the gas in equilibrium with the capillary blood before and after the experiments is calculated from the CO concentration in the rebreathing bag, assuming F_c is proportional to F_{cO_2}. The values are: 0.0006 per cent initially; 0.0055 per cent finally. The average increment of F_c per experiment is 0.0007 per cent CO. The average F_c in each experiment can thus be computed. (i) The mixed expired alveolar CO concentration in per cent (dry) corrected for the pressure of COHb in the capillary blood by subtracting F_c (Col. h) from $\overline{F_{AE}}$ (Col. f). (j) The mixed expired alveolar CO concentration corrected for the presence of COHb in the capillary blood is now adjusted to an initial alveolar CO concentration of 0.2 per cent (dry) by multiplying by $\frac{0.2}{\text{initial CO con.}}$ or $\frac{0.2}{\text{Col. e}}$.

Comparison of figures in Column g and Column j shows that the correction applied for the mixed venous COHb content changes the final value for $\overline{F_{AE}}$ by only a small amount.

probably had more CO in it at time zero, before any CO was absorbed, than the final sample did. This fact would increase the difference in CO concentration between the two samples and thus would increase the calculated value for D. This type of error is minimized in the present experiments by comparing the CO concentration in a given sample at any time with the initial CO concentration in *that* sample, using He as an insoluble tracer. It must be emphasized that although unevenness of ventilation can alter the absolute concentration of CO in a given fraction of the expired breath, it cannot *per se* produce a curvature of the plot of the logarithm of CO concentration against time.

A number of possible causes of the non-linearity of the semi-log plot of the mixed expired alveolar CO concentration ($\overline{F_{AE}}$) will be discussed:

1. *Systematic error in measurement.* The possible error in the measurement of time must have been less than 1 second because inspiration and expiration together took less than 2 seconds and time measurements were made in all cases from the start of inspiration to the start of expiration. Inspection of Figure 3 shows that an error of one second could not straighten the curve appreciably.

The ratio $\frac{F_{I_{He}}}{\overline{F_{AE_{He}}}}$ was found to be independent of the length of time the breath was held within limits of measurement. Thus the only analysis that could have produced the upward concavity of the log $\overline{F_{AE}}$ plot was that of $\overline{F_{AE}}$ itself.

The analytical error in $\overline{F_{AE}}$ ranged from ±2 per cent early in the disappearance curve to a maximum of ±10 per cent in the 50 second region, as stated earlier. If this ±10 per cent error is applied to the data in Figure 3, in any order whatsoever in disregard of its probably random distribution, the slope can hardly be changed more than 25 per cent in any region. Since the slope varies two to five fold over the

curve, the analytical errors alone could not produce the curvature found.

2. *Saturation of tissues with dissolved CO.* If a significant fraction of the CO absorbed during the early part of the disappearance curve were dissolved in tissues exposed to the pulmonary gases, as these tissues became saturated with CO, the total rate of CO disappearance would decrease. This phenomenon would produce a curvature upwards of the type seen in Figure 3. Assuming a total volume of tissue exposed to alveolar gas of only 1,000 ml. (5), since in this instance our interest is limited to tissue that is exposed to alveolar gas and could become saturated with CO, with a total lung gas volume of 4,000 ml. and a partition coefficient for CO of 0.02 (6), at equilibrium the tissue would contain $\frac{1{,}000 \times 0.02}{4{,}000}$ or 0.5 per cent of the CO contained in the alveolar gas. Even if the pulmonary tissue equilibrated with the *initial* alveolar gas concentration and released it at a time when the alveolar CO concentration had decreased to low levels, the increase in the alveolar CO concentration could not be more than 0.5 per cent of the initial value. Inspection of Figure 3 shows that 0.5 per cent of 0.2 per cent CO, or a 0.001 CO change in $\overline{F_{AE}}$ would not substantially change the slope of the curve.

3. *Effect of decreasing lung volume.* During breathholding, the volume of gas in the lung will decrease since CO_2 will be added at a slower rate than oxygen will be removed. This decrease in volume will tend to increase the concentration of CO in the lung gas as time proceeds. However, the total decrease in lung volume during one minute of breathholding is unlikely to exceed 5 to 10 per cent (8), which would produce a proportional increase in CO concentration. It can be seen from Figure 3 that this factor is far too small to produce the curvature observed.

4. *Changes in total alveolar pressure.* The rate of disappearance of CO from the alveolar gases is proportional to its partial pressure, so that an increase in total intrapulmonic pressure produces an increase in the rate of CO loss. If the subjects by thoracic effort produced a higher intrapulmonic pressure early in the period of breathholding than later, the exponential rate of CO disappearance would decrease with time and an upward curvature of log $\overline{F_{AE}}$ would result. According to the results shown in Figure 4, a change of 80 mm. Hg in the intrapulmonic pressure during breathholding did not alter the rate of CO disappearance noticeably. Since the subjects were trying to relax during breathholding under the usual experimental conditions, the intrapulmonic pressure would not have been expected to rise much above 22 mm. Hg (9). It has therefore been concluded that changes in intrapulmonic pressure probably did not cause the curvature seen in Figure 3.

5. *Diffusion of CO into the alveoli from the dead space.* The concentration of CO in the alveolar gas decreases markedly during the period the breath is held. There will consequently be a tendency for CO to diffuse into the alveoli from the dead space. Experiments were performed on two subjects in which several liters of the inspired gas mixture containing CO were followed without interruption by a liter of air so that the dead space was washed free of CO (10). Under these circumstances, the shape of log $\overline{F_{AE}}$ versus time was unchanged. It was therefore concluded that this factor was unimportant in producing the curvature.

6. *Absorption of CO from the inspired gas before it has mixed completely with gases already in the lungs.* Some CO may be absorbed at a higher rate from the inspired gas, rich in CO, before it is completely mixed with gas initially in the lungs. However, it seems likely that mixing of gas within an alveolus is complete within a few thousandths of a second following the completion of inspiration (11). Therefore, this factor cannot be responsible for the curvature after the first second. In addition, there are apparently no sharp discontinuities in the different curves between the 5 second point and the time zero point (Figure 3, for example). Since the latter point is calculated on the assumption of complete mixing of the inspired gas with the residual gas in the lungs before any absorption takes place, it seems reasonable to conclude that this is approximately true.

7. *Presence of significant carboxyhemoglobin in the capillary blood.* When there is COHb in the capillary blood, the alveolar CO concentration will fall to approach a value in equilibrium with it at an oxygen tension and O_2Hb saturation representing an average along the pulmonary capillary. If the presence of the COHb is ig-

nored, an apparent upward curvature of log $\overline{F_{AE}}$ will result. COHb can be present in the pulmonary capillary blood either because it is in the mixed venous blood, or because it is formed in the pulmonary capillary. In either case, the blood COHb can be taken into account by plotting log $(\overline{F_{AE}} - F_c)$ against time instead of log $\overline{F_{AE}}$, where F_c is the concentration of CO in equilibrium with the blood under average pulmonary capillary conditions. This procedure is justified by Equation *17* in the accompanying paper (4).

Considering first the possibility that there was significant COHb in the mixed venous blood, it is interesting to note that it was impossible to choose any value of F_c which would produce a linear plot of log $(\overline{F_{AE}} - F_c)$ for most of the data. Although the highest COHb concentration found in the mixed venous blood of smokers (12) could not explain an upward curvature of the degree seen in Figure 3, estimates of the mean capillary COHb concentration were made before and after a series of experiments in four normal subjects, and values for capillary COHb calculated by interpolation. In three of the subjects, F_c was estimated by the method of Siösteen and Sjöstrand (13), which consisted of a preliminary 4 minute washout of the lungs with 100 per cent O_2 followed by 4 minutes of rebreathing in a 6 liter closed circuit filled with 100 per cent O_2 and including a CO_2 absorber. The final CO concentration was considered F_c at the final O_2 concentration in the closed circuit. This value of F_c was corrected to the actual capillary O_2 tension during the breathholding experiments, by considering F_c proportional to the capillary O_2 tension. In a fourth subject, these estimates of F_c were checked by the blood COHb method of Roughton and Root (14) assuming a Haldane coefficient of 220, which agreed within less than 10 per cent.

Table I presents the data and computations of such a series of experiments in which the alveolar CO concentration was corrected for the presence of the COHb in the capillary blood. The uncorrected data (Col. g) are plotted in Figure 4 as an example of normal uncontrolled intrapulmonary pressure during breathholding. A comparison of $\overline{F_{AE}}$ uncorrected (Col. g) with $\overline{F_{AE}}$ corrected (Col. g) shows that the effect of the blood COHb concentration is small and could not be responsible for the curvature seen in Figure 4. The increase in effective CO gas concentration in equilibrium with the blood (F_c) (Col. (h) 0.0055 per cent CO) during a complete series of experiments is so small as to exert a minimal effect on the curvature of the log $\overline{F_{AE}}$ plot against time. Results were similar in the three other normal subjects. Therefore, it can be concluded that the curvature of the log $\overline{F_{AE}}$ plot is not due mainly to the presence of COHb in the mixed venous blood. However, as a precaution in all series of experiment, the individual experiments were done in the order 60, 10, 50, 20, 40, and 30 seconds to minimize any effect of an increasing mixed venous COHb concentration upon the CO disappearance curve.

There remains the possibility that sufficient COHb forms during a single passage through the alveolus to interfere with the further uptake of CO, either in the lung as a whole, or in certain alveoli. Although the data of Table I indicate that there is no marked rise in the total blood COHb, it must be remembered that large amounts of COHb could have been formed over short periods of time, which when diluted by the total blood volume appear insignificant. It is possible to make a calculation of the ratio of the blood CO tension at the end of the capillary (P_c) to the alveolar CO tension (P_A). A maximal value for the CO diffusing into the capillary equals pulmonary diffusing capacity (D in ml. gas/sec. × mm. Hg) × alveolar CO tension (P_A in mm. Hg). This is maximal because it neglects the presence of CO tension in the blood, either from COHb or from the slowness of the combination of CO with Hb. $\frac{P_A D}{Q}$ where Q is the pulmonary blood flow in ml. per sec., gives the change in COHb concentration in one passage through the lungs in ml. gas per ml. blood. The increase in the capillary CO tension due to this COHb can be computed approximately from the Haldane relation, $P_c = \frac{P_{cO_2}COHb}{210\,O_2Hb}$, where P_{cO_2} is the end capillary O_2 tension in mm. Hg and COHb and O_2Hb are the respective concentrations of the compounds in ml. gas per ml. blood. Thus $P_c = \frac{P_A D P_{cO_2}}{Q\,210\,O_2Hb}$ and $\frac{P_c}{P_A} = \frac{D P_{cO_2}}{Q\,210\,O_2Hb}$.

Under the most severe circumstances, while

breathing 100 per cent O_2, with D certainly no greater than 30 ml. per min. × mm. Hg or 0.5 ml. per sec. × mm. Hg, Q equal to 100 ml. per sec., P_{cO_2} equal to 650 mm. Hg, and O_2Hb equal to 0.19 ml. gas per ml. blood, $\frac{P_c}{P_A} = \frac{0.5 \times 650}{100 \times 210 \times .19} = 0.082$. Therefore, the CO tension at the arterial end of the pulmonary capillary would be 8 per cent of the alveolar CO tension. Even this large an equilibrium tension of CO in the capillary blood could not cause the upward curvature of the plot of log ($\overline{F_{AE}}$) (inspiring 90 per cent O_2) in Figure 3. Actually, 8 per cent is much too high a figure for P_c/P_A because (a) with a high alveolar O_2 concentration the slowness of the reaction of CO with Hb would decrease the effective value of D by at least one-half and (b) the average P_c/P_A along the capillary would be about one-half that at the end of the capillary. Furthermore, regardless of the absolute value of the average tension of CO resulting from an increase in COHb during passage through the alveolus, it would tend to be proportional to the alveolar CO concentration, and would not produce the curvature of Figure 3. Thus it seems possible to rule out an increase in capillary COHb during one passage through the alveoli as a cause of the failure of the mean mixed expired alveolar CO concentration to fall exponentially as regards the lung as a whole. However, there remains a more subtle possibility that in some parts of the lung, the blood flow is so slow, or the diffusing capacity so large, that a significant increase in COHb during a single passage through the alveoli does take place. Under these circumstances the rate of disappearance of alveolar CO would probably be exponential, but would be less than that of the rest of the lung. The true mixed expired alveolar CO concentration being the average of at least two exponential processes under these conditions would show an upward curvature as seen in Figure 3. This possibility cannot be completely ruled out at this time, but can hardly have been occurring in a significant part of the lung, since the measured increase in the total blood COHb is so small.

8. *Chemical action of CO on the alveolar membrane.* At the present time the movement of gases across the pulmonary membrane is considered a passive process. No evidence is available that CO interferes with diffusion by altering the diffusing characteristics of the alveolar membrane.

9. *Variation in the exponential constant during the breathholding period.* Examination of Equation *2* in the accompanying paper (4) shows that the exponential constant is $\left[\frac{-DP_bCt}{V_A}\right]$, where C is a correction factor allowing for the slowness of the rate of combination of CO with Hb. Anything that varied the total value of this expression would vary the rate of decrease of the alveolar CO concentration. The alveolar volume (V_A) was certainly constant during the experiment, as was the barometric pressure (P_b). Because the rate of combination of CO and Hb increases with a decrease in O_2 tension, the tension of CO in the capillary blood resulting from the slowness of this reaction would have decreased with time during breathholding. C would therefore have increased, that is approached unity, as the breathholding continued, which would have had the effect of increasing the rate of fall of alveolar CO concentration, and the plots of Figure 3 should have curved *downwards*. Variation in alveolar O_2 tension cannot therefore have been a cause of the upward curvature found.

There remains the possibility that the pulmonary diffusing capacity (D) decreased during the period of breathholding. It seems unlikely that the specific diffusing characteristics of the tissue composing the alveolar membrane will change greatly in a minute. However, changes in the number, length and distension of the pulmonary capillaries might occur during breathholding and would alter D. For instance, it has been reported (15) that immediately after taking a deep breath the blood flow through the lung first increases greatly, and then gradually decreases as the breath is held. D might be expected to parallel these changes in pulmonary blood flow. This would cause the decay exponent (Equation *2*) to decrease with time during the period of observation and the log $\overline{F_{AE}}$ versus time plot would curve upwards. However, to explain the degree of curvature found D would have to alter sufficiently to decrease the decay exponent by one-third to one-fifth during the 60 seconds of breathholding. Furthermore, the increased blood flow as indicated by Armitage and Arnott's (15) technique subsided after 10 seconds of breathholding while the curvature of the CO

disappearance curves continues out as far as 30 to 40 seconds. One would expect that any change in the diffusing capacity of the lung produced by a maximal inspiration acting through a mechanism such as this would be diminished if smaller and slower breaths were taken. However, in experiments on four normal subjects, inspiration was started from functional residual capacity instead of residual volume, the inspired gas volume was reduced from the usual average of 4 liters to between 600 and 900 ml., and the inspiration was made more slowly than in the case of the larger inspired volumes. The total lung volume during breathholding was the same as in the usual experiments, *i.e.*, approximately maximal. No marked difference was observed between the curves obtained under these conditions and those in which deeper breaths were taken, suggesting that the expansion of the lungs is not an important factor in producing the curvature of the log $\overline{F_{AE}}$ plot.

Several additional experiments were done on one subject to get more clearcut evidence on this point. The subject inspired almost maximally from residual volume, as in the experiments described under Methods, except that he inspired air. He held this volume for 40 seconds and then made a further gentle inspiration of approximately 80 ml. of a mixture of 88 per cent He and 12 per cent CO. This second volume (as well as the first volume) was held for 5 to 15 seconds, and then expired, an alveolar sample being collected as described before. The initial CO concentration was calculated from the He dilution as with the other experiments, the high inspired concentrations of CO giving initial alveolar CO concentrations from 0.1 to 0.2 per cent.[11] If the pulmonary diffusing capacity were decreasing with time during breathholding, or some similar process were going on, the rate of CO disappearance at 40 seconds in this experiment should have been less than the rate of CO disappearance at the start of breathholding, as demonstrated in Figure 3, since we assume that the inspiration with minimal inspiratory effort of 80 ml. into a lung which already contained more than 5 liters would not significantly alter D. However, the actual disappearance rate in these experiments was considerably *greater* than the CO disappearance rate at the start of breathholding breathing air. This is reasonable because of the fall in alveolar O_2 tension which would cause a rise in the CO uptake (7), but is incompatible with the idea that a large decrease in D occurs during the period of breathholding. The possibility that the increase in alveolar CO_2 concentration and/or the decrease in alveolar O_2 concentration with breathholding might produce a gradual decrease in D by a direct chemical effect on the pulmonary vessels, is minimized by the experiment just described. Thus while D might decrease by one-half or one-fifth during the 60-minute period of breathholding, it appears very unlikely.

In summary, up to this point a number of possible explanations for the upward curvature of the plot of log $\overline{F_{AE}}$ against time have been presented and it has been concluded that no one of them is acceptable as a major cause of the effect on the basis of present knowledge. If all the mechanisms enumerated above operated together to produce an upward curvature of the log $\overline{F_{AE}}$ plot, the points would still not fall on a line.

10. *The existence of significantly different diffusing phases in the lung.* As had been previously demonstrated (4) the logarithm of mixed expired alveolar CO concentration ($\overline{F_{AE}}$) plotted against the time will not be a straight line but will curve upwards unless all the exponential constants $\left[\frac{-D_iPbC_i}{V_{A_i}}\right]$ are equal in all phases. $\frac{D_i}{V_{A_i}}$, the ratio between diffusing capacity and volume in an alveolus, is probably related to the number and degree of distension of the pulmonary capillaries. Since the number of capillaries per alveolus varies throughout the lung (17) and the hydrostatic (distending) component of capillary pressure is greater at the base of the lung than at the apex, it would be remarkable if the exponential constants were equal in all parts of the lung. A four- or five-fold variation in $\frac{D}{V_A}$ throughout the lung is not unreasonable and at the present time this is considered the most likely single cause of the curvature of the plot of log $\overline{F_{AE}}$ against time.

[11] Inspired gas appears in the expired alveolar gas after inspirations of as little as 40 ml. (16).

If $\frac{D}{V_A}$ varies throughout the lung, the estimation of the total D of the lung demands precise knowledge of the V_A associated with each D. In other words, the lung would have to be broken up into discrete "diffusing phases." Although the $\overline{F_{AE}}$ curves can be separated into two exponential decay terms, and two "diffusion phases" can be computed, it seems more likely at the present time that there is a statistical distribution of $\frac{D}{V_A}$ throughout the normal lung. This makes the estimation of the total lung diffusing capacity very formidable. A question might even be raised as to the practical value of total D, since it is independent of the ventilation, and a poorly ventilated region of the lung with a large D would not be so effective as would appear from total D alone.

In the preceding paper (4) it was pointed out that if the ratio of diffusing capacity to effective ventilation $\left(\frac{D}{V_T - V_D}\right)$ throughout the lung is not constant, any "steady state" method of estimating the true total diffusing capacity of the lung will be in error. However, $\frac{D}{V_A}$ can vary throughout the lung without $\frac{D}{V_T - V_D}$ of necessity varying.

At present the difficulties of measuring the true total diffusing capacity in the presence of varying ratios of $\frac{D}{V_A}$ and/or $\frac{D}{V_T - V_D}$ are so formidable that methods based on the measurement of an overall or "apparent" total D will have to continue to be used. However, the fact that the "apparent" total D so measured is influenced by factors other than the diffusing capacity of the lung, must be remembered when differences in this measurement are interpreted in terms of pathological change.

The uptake of chemically inert gases from the lungs has been recently discussed by Kety (18). The relationships presented by him can be expressed in terms of different perfusion phases, that is phases with different blood flow per unit alveolar volume, or per unit alveolar ventilation. It may be found necessary to use this approach to reconcile experiment and theory.

SUMMARY

1. The disappearance of CO from the alveolar gas of the lung during breathholding has been investigated in seven normal subjects.

2. The alveolar CO concentration did not fall exponentially with time as had been assumed by previous workers.

3. The most likely single explanation of this phenomenon is that the diffusing capacity per unit gas volume varies throughout the lung.

4. This finding is relevant to the consideration of the validity of present methods of measuring the diffusing capacity of the lungs.

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A STUDY OF MINUTE TO MINUTE CHANGES OF ARTERIO-VENOUS OXYGEN CONTENT DIFFERENCE, OXYGEN UPTAKE AND CARDIAC OUTPUT AND RATE OF ACHIEVEMENT OF A STEADY STATE DURING EXERCISE IN RHEUMATIC HEART DISEASE

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When the Fick equation for the estimation of the cardiac output is applied to the values obtained from a few mixed venous blood samples drawn during a period of measurement of oxygen uptake, they should be truly representative of the values throughout that period. This is most unlikely to be the case unless the subject is in a steady state. The Fick principle with cardiac catheterization has frequently been employed to measure the cardiac output of patients with rheumatic heart disease during exercise, the measurements being made after an arbitrary period varying from two to ten minutes, after which it has been hoped, but never proved, that a steady state had been reached, even in patients with gross disability. Cournand (1), who has at all times emphasized these difficulties, considers the steadiness of the oxygen consumption, of the respiratory quotient and of the pulse rate useful criteria of constant levels of blood oxygenation and cardiac output.

In the work reported here the standard Fick method has been modified and mixed venous and arterial blood samples have been collected frequently throughout a period of exercise and recovery in a number of patients with rheumatic heart disease, mainly involving the mitral valve. The percentage saturation of the mixed venous blood entering the lungs can be closely followed by the technique described even in a rapidly changing state. However, if the mean (in time) A–V difference [1] is employed in the Fick formula to calculate the cardiac output while both the A–V difference and blood flow are changing then some error will ensue. The magnitude and importance of such errors will be discussed later in relation to the results. Particular attention has been paid to the rate of achievement of a steady hemodynamic state during exercise. A similar series of studies has been carried out on normal subjects, which although briefly mentioned for comparative purposes, is being reported fully elsewhere (2).

[1] Throughout this article "A–V difference" is used to denote the arterio-venous oxygen content difference.

SUBJECTS, METHODS AND PROCEDURE

The sixteen subjects studied had been presented for consideration of mitral valvulotomy. Ten of these patients had mitral stenosis (M.S.), two mitral stenosis and mitral regurgitation (M.S. & R.), two predominant mitral regurgitation (M.R.), and two mitral and aortic stenosis (M. & A.S.) None had evidence of active rheumatism or of congestive failure. All were taking digitalis with the exception of cases C1, C7, and C10. Clinical data concerning each patient is given in the Appendix. There were a number of important modifications of the standard methods.

Blood oxygenation and arterio-venous oxygen content difference

The almost continuous observation of the arterio-venous oxygen content difference by the sampling of arterial and mixed venous blood at frequent intervals necessitated the rapid and accurate analysis of very large numbers of blood samples and a spectrophotometric method was therefore developed. Briefly, 2 ml. samples of blood were anaerobically diluted (about 1 in 5) with a hemolyzing solution and an aliquot of this solution placed in sample tubes made from ordinary glass tubing, the remainder being set aside for oxygenation. These tubes were marked and the same light path employed throughout. The optical density of the tube and solution was then determined using a standard Unicam spectrophotometer. The optical density was again determined after complete reduction of the hemoglobin by a drop of saturated sodium hydrosulphite solution. The tube was then cleaned and filled with the oxygenated solution, and the optical density determined once more. The percentage saturation was then easily calculated. The oxygen capacity of the blood sampled at rest and during exercise was measured by the Van Slyke manometric or standard photometric methods and thus the oxygen content of the blood samples determined. A duplicate estimation of percentage saturation was carried out on all blood samples. The technique and accuracy, which is most satisfactory as

compared with standard Van Slyke estimations, has been reported elsewhere (3). The small size of the individual blood samples allowed frequent sampling without undue blood loss.

Ventilation and oxygen uptake

During exercise and recovery the expired air was collected in a Tissot spirometer. A tracing was taken which was interrupted by a minute signal and during each minute the expired air was sampled at a constant rate by mercury siphoning from a side arm near the spirometer and separated from the mouthpiece by 3 feet of corrugated tubing. Haldane gas sampling tubes of 50 ml. capacity were employed and only a few seconds at the end and beginning of each minute were needed to change the gas sampling tubes. Gas samples were analysed for oxygen and carbon dioxide content by the Scholander micromethod and duplicate analyses were required to check by 0.03 per cent. It was appreciated that simultaneous changes in ventilation and oxygen extraction would cause some error in the determination of oxygen uptake by this method and this is discussed further in the section dealing with sampling errors. A series of experiments was carried out on patients with comparable cardiac disability in which the oxygen uptake over a minute was measured simultaneously by both side-arm sampling and by the standard technique of sampling from the Tissot spirometer. These results were compared in 21 instances, measurements being made during a steady state of exercise, in the first minute of exercise and in the first minute of recovery. The results obtained are shown in Figure 1.

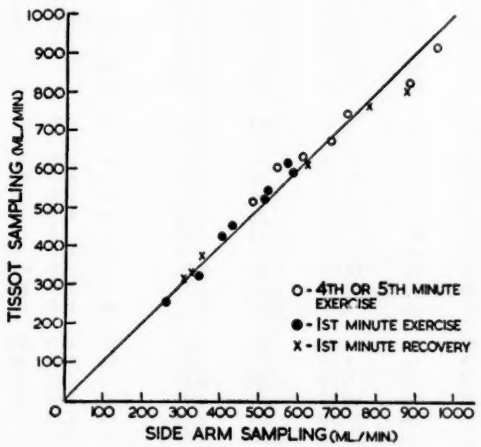

FIG. 1. COMPARISON OF SIMULTANEOUS OXYGEN UPTAKE MEASUREMENTS (N.T.P.), BY CONTINUOUS SIDE ARM AND BY SPIROMETER SAMPLING, DURING EXERCISE AND RECOVERY, IN PATIENTS WITH HEART DISEASE

The coefficient of correlation between the two series of measurements is $r = 0.99$ $(n = 21)$ and the standard difference within pairs of measurements is 21.8 ml.

Although the error is small and non-systematic, if the authors were repeating this type of study they would favour the use of a number of 50 liters-capacity, light rubberized gaberdine "Douglas" bags so that each minute's expirate could be collected separately and measured and analysed after the experiment.

Exercise

Great care was taken to ensure a constant level of work. The subjects exercised in the horizontal position on a bicycle ergometer fixed to the foot of the screening table. The shoulders were steadied by supports so that the subject was able to bear easily and comfortably on the pedals. Above the apparatus was a simple differential indicator, one side being geared to the flywheel and the other to a gramophone motor with the governor lever in a fixed position. The patient and all members of the team could watch the movements of the differential arm and thus ensure a steady rate of pedalling. The degree of work was varied from subject to subject by altering the tension of the friction belt on the flywheel. All subjects were shown the apparatus the day before the study, and, with little practice, were able to pedal at a steady rate. In each case the maximum degree of work which could be sustained for five minutes without undue distress was chosen. Thus all the studies were at levels of exercise close to the tolerance of the patient.

Pressure recording

Vascular pressures were recorded by means of capacitance manometers. The degree of damping and conditions of adequate manometric performance were as previously described (4). The manometric zero was fixed 10 cm. above the table on which the patient was lying. The zero setting and calibration of the electromanometer were checked frequently, particularly before and after any important pressure measurements.

Procedure

Each subject was given 0.4 Gm. of quinidine sulphate by mouth three hours before the procedure; 0.13 to 0.2 Gm. of sodium amytal were also given two and one hours previously. No meal had been taken for six hours but 50 Gm. of glucose in a fruit drink were given half an hour before the catheterization. The resting cardiac output was determined and pressure measurements made fifteen minutes after the tip of the catheter had been passed into the pulmonary artery and an indwelling arterial needle inserted into the brachial artery, providing the patient was settled and the minute ventilation steady. During the three-minute period of expired air collection, three 3 ml. samples of mixed venous and arterial blood were taken.

Thirty seconds before the commencement of exercise a sample of mixed venous blood was taken. Fifteen seconds before, the legs were raised and the feet steadied on the pedals. Five seconds before, the pedals were started by team members to overcome the initial inertia and to achieve rapidly the required rate of pedalling.

TABLE I

Observations at rest and during five minutes exercise and recovery

Subject	Minute of study*	O_2 Consum. (*ml./min./m.²*) N.T.P.	A-V diff. (*vols. %*) N.T.P.	C.I. (*L./min./m.²*)	Heart rate	Stroke vol. (*ml.*)	Vent. (*L./min./m.²*) N.T.P.	R.Q.	% O_2 extn.	Pressure (*mm. Hg*) Mean P.A.	B.A. (S)	B.A. (D)
C1	Rest	160	3.78	4.21	90	79	3.33	.79	4.78	20	110	78
	Ex. 1	330	5.63	5.85	112	88	6.68	.85	4.93	—	—	—
SA = 1.68 m.²	2	524	8.88	5.90	124	80	8.64	.75	6.06	—	—	—
RC = 21.10†	3	602	8.88	6.79	129	88	9.30	.77	6.48	—	—	—
EC = 21.65	4	564	8.88	6.36	135	79	9.30	.85	6.07	—	—	—
	5	548	8.88	6.17	134	77	9.13	.84	6.00	30	—	—
	Ry. 2	220	5.85	3.75	—	—	5.87	1.08	3.74	—	—	—
	5	142	3.25	4.35	—	—	4.08	1.04	3.47	17	—	—
C2	Rest	176	5.11	3.44	84	66	5.62	1.06	3.12	29	112	64
	Ex. 1	332	5.85	5.67	104	87	7.91	.92	4.20	—	160	90
SA = 1.60 m.²	2	511	9.83	5.19	122	68	10.54	.83	4.93	—	160	90
RC = 16.70	3	696	10.19	6.83	135	81	14.06	.93	4.95	—	160	88
EC = 18.20	4	710	10.10	7.03	140	80	14.76	.97	4.81	66	152	82
	5	742	9.28	7.99	136	94	15.81	1.02	4.69	50	144	84
	Ry. 2	373	5.92	6.79	114	88	12.92	1.36	2.84	—	130	78
	5	191	5.82	3.29	111	47	7.32	1.22	2.59	35	100	68
C3	Rest	108	5.10	2.12	66	51	3.32	.84	3.26	38	140	70
	Ex. 1	209	9.17	2.28	106	34	6.43	.88	3.24	—	140	94
SA = 1.60 m.²	2	516	14.43	3.58	138	41	12.25	.83	4.21	—	140	96
RC = 18.80	3	493	15.99	3.08	153	32	16.31	1.09	3.02	—	148	96
EC = 19.50	4	628	15.31	4.10	153	43	18.69	1.07	3.36	65	140	80
	5	538	15.41	3.49	—	—	16.75	1.07	3.21	—	—	—
	Ry. 1	379	12.09	3.13	78	64	10.31	1.04	3.67	54	155	80
	2	214	6.92	3.09	79	63	8.03	1.38	2.67	—	—	—
	3	167	6.24	2.68	82	52	6.35	1.34	2.63	—	130	70
	4	151	5.66	2.67	87	49	5.59	1.28	2.69	—	120	65
	5	147	4.97	2.95	84	56	5.50	1.18	2.67	45	125	70
C4	Rest	134	4.49	2.99	67	66	4.06	.89	3.30	35	104	56
	Ex. 1	298	6.39	4.66	102	68	10.09	1.07	2.95	—	—	—
SA = 1.49 m.²	2	476	10.12	4.70	132	53	12.56	.84	3.79	—	124	68
RC = 18.25	3	596	10.65	5.60	146	57	18.28	1.00	3.26	—	144	72
EC = 17.75	4	552	10.56	5.22	149	52	20.00	1.08	2.76	65	144	72
	5	516	10.83	4.77	—	—	20.00	1.14	2.58	—	—	—
	Ry. 2	232	5.95	3.91	90	65	9.71	1.25	2.39	40	—	—
	5	149	4.79	3.11	80	58	5.23	.99	2.84	35	—	—
C5	Rest	163	5.59	2.93	87	54	4.53	.93	3.61	40	100	62
	Ex. 1	284	8.72	3.27	129	41	7.53	.91	3.78	—	120	80
SA = 1.61 m.²	2	498	13.24	3.76	158	38	13.14	.94	3.79	—	120	80
RC = 20.11	3	606	14.35	4.22	168	41	16.83	1.18	3.60	—	120	80
EC = 21.26	4	642	15.03	4.27	178	37	19.20	1.26	3.35	55	120	80
	5	663	16.05	4.13	175	38	20.85	1.26	3.18	—	120	80
	Ry. 1	564	13.54	4.17	163	41	15.67	1.21	3.60	—	95	70
	2	333	8.61	3.87	143	44	14.55	1.71	2.29	60	110	70
	3	237	7.29	3.25	110	48	12.09	1.67	1.96	—	100	65
	4	204	6.80	3.00	85	57	10.68	1.54	1.91	—	100	60
	5	197	6.59	2.99	80	60	9.29	1.39	2.12	45	100	60
C6	Rest	136	5.22	2.59	72	55	3.80	.92	3.56	32	110	60
	Ex. 1	180	6.51	2.77	104	41	5.60	.86	3.22	—	130	70
SA = 1.52 m.²	2	439	11.58	3.80	120	48	9.75	.85	4.51	—	135	75
RC = 19.86	3	447	12.57	3.56	135	40	10.47	.90	4.27	—	120	70
EC = 20.61	4	450	13.29	3.39	135	38	11.30	.96	3.98	66	120	75
	5	445	13.60	3.28	—	—	12.23	1.05	3.64	—	—	—
	Ry. 1	415	12.12	3.43	88	59	13.90	1.09	2.99	—	140	65
	2	226	7.38	3.05	85	55	10.78	1.46	2.09	45	100	60
	3	159	6.18	2.57	85	46	7.47	1.32	2.13	—	96	58
	4	137	6.02	2.28	88	39	6.22	1.24	2.20	—	90	56
	5	147	6.25	2.36	88	41	5.90	1.11	2.49	38	98	60

* Ex = Exercise, Ry = Recovery.

† RC and EC = Resting and Exercising blood oxygen capacity (vols. %).

TABLE I—*Continued*

Subject	Minute of study*	O_2 Consum. (*ml./min./m.²*) N.T.P.	A-V diff. (*vols. %*) N.T.P.	C.I. (*L./min./m.²*)	Heart rate	Stroke vol. (*ml.*)	Vent. (*L./min./m.²*) N.T.P.	R.Q.	% O_2 extn.	Pressure (*mm. Hg*) Mean P.A.	B.A. (S)	B.A. (D)
C7	Rest	141	3.92	3.61	95	60	6.02	.94	2.35	65	100	60
	Ex. 1	209	5.28	4.16	118	56	9.66	.90	2.17	—	110	66
SA = 1.58 m.²	2	301	8.06	3.74	133	44	14.63	.90	2.06	—	115	70
RC = 19.24	3	347	9.22	3.77	140	43	18.97	1.01	1.83	—	112	70
EC = 19.39	4	377	9.35	4.08	146	44	20.68	1.07	1.82	85	112	70
	5	411	9.50	4.33	147	47	22.84	1.09	1.80	—	114	72
	Ry. 1	375	8.64	4.34	143	48	19.05	1.05	1.97	—	110	70
	2	238	5.53	4.30	129	53	13.46	1.02	1.77	73	108	60
	5	151	3.99	3.77	99	60	7.87	.98	1.91	62	106	60
C8	Rest	140	4.96	2.83	72	64	4.26	.90	3.30	48	100	60
	Ex. 1	240	7.60	3.15	94	55	6.73	.84	3.56	—	120	75
SA = 1.64 m.²	2	317	10.78	2.94	113	43	8.11	.79	3.91	—	120	75
RC = 19.30	3	361	11.86	3.04	120	42	10.15	.85	3.56	—	—	—
EC = 19.60	4	334	12.56	2.66	120	36	9.84	.87	3.39	25‡	124	72
	5	358	12.94	2.77	125	36	10.53	.92	3.40	—	120	68
	Ry. 2	201	5.62	3.59	92	64	5.96	.97	3.38	18‡	106	62
	3	164	3.47	4.72	89	87	5.27	1.04	3.11	—	100	60
	4	142	3.33	4.27	85	82	4.86	1.03	2.94	—	100	60
	5	131	3.72	3.53	84	69	4.49	1.01	2.92	18‡	102	62
C9	Rest	146	4.82	3.12	82	53	4.74	.98	3.06	52	100	60
	Ex. 1	253	7.59	3.33	123	39	9.51	1.04	2.66	—	130	70
SA = 1.45 m.²	2	357	12.10	2.95	153	28	13.35	.98	2.67	—	130	70
RC = 18.84	3	397	13.08	3.18	165	28	17.54	1.10	2.37	—	130	70
EC = 20.24	4	474	14.21	3.33	168	29	19.84	1.10	2.39	83	130	70
	5	494	14.97	3.30	163	29	21.14	1.16	2.34	—	120	70
	Ry. 1	394	12.87	3.06	155	29	15.86	1.14	2.49	—	110	58
	2	234	8.34	2.95	130	33	11.96	1.27	2.05	80	120	70
	3	215	7.16	3.01	108	40	9.08	1.18	2.37	—	106	66
	4	199	6.44	3.08	103	43	7.68	1.07	2.59	—	—	—
	5	183	6.11	3.00	100	44	6.78	1.04	2.71	76	100	62
C10	Rest	184	4.76	3.86	108	57	6.25	.93	2.94	65	110	62
	Ex. 1	328	6.72	4.89	122	64	14.20	1.09	2.31	—	104	56
SA = 1.59 m.²	2	355	8.80	4.04	138	47	20.54	1.33	1.73	—	100	52
RC = 11.05	3	430	9.29	4.62	148	50	24.53	1.43	1.75	—	88	56
EC = 12.05	4	418	9.74	4.29	150	46	25.62	1.49	1.63	78	86	52
	Ry. 1	382	10.28	3.71	148	40	22.45	1.44	1.70	80	104	52
	2	360	8.86	4.07	143	45	20.37	1.44	1.77	—	132	68
	3	297	6.51	4.56	133	55	17.47	1.42	1.70	80	146	72
	4	270	5.25	5.16	123	67	15.20	1.38	1.78	—	146	72
	5	208	4.70	4.43	115	61	12.30	1.34	1.69	—	140	72
	6	177	5.30	3.33	110	48	10.32	1.26	1.71	78	140	70
C11	Rest	149	5.26	2.84	115	36	5.05	.89	2.96	50	100	60
	Ex. 1	227	7.47	3.05	138	32	8.09	.88	2.81	—	110	76
SA = 1.46 m.²	2	318	9.64	3.06	160	28	10.31	.83	3.08	—	110	76
RC = 18.00	3	335	10.77	3.11	168	27	12.23	.96	2.74	—	110	76
EC = 18.90	4	345	11.15	3.10	158	29	13.28	1.04	2.60	75	114	78
	5	366	11.06	3.32	153	32	13.67	1.03	2.68	—	—	—
	Ry. 1	301	9.64	3.12	148	31	10.50	1.00	2.87	69	—	—
	2	190	6.71	2.84	139	30	8.18	1.09	2.33	60	102	76
	3	163	5.67	2.88	129	33	6.90	1.09	2.36	—	100	80
	4	142	6.24	2.27	110	30	6.68	1.09	2.12	—	—	—
	5	147	5.95	2.47	102	35	6.16	1.05	2.39	56	102	76
C12§	Rest	162	6.20	2.61	115	39	5.24	.97	3.09	65	130	80
	Ex. 1	230	8.17	2.82	140	34	7.78	.94	2.96	72	—	—
SA = 1.70 m.²	2	312	10.25	3.05	148	35	9.35	.90	3.34	—	—	—
RC = 18.35	3	349	11.28	3.09	150	35	12.25	.94	2.85	80	—	—
EC = 19.45	4	344	11.86	2.90	155	32	13.23	1.01	2.60	—	130	80
	5	364	12.06	3.01	155	33	13.41	1.00	2.71	—	—	—
	Ry. 2	241	9.82	2.46	100	42	8.94	1.01	2.70	70	—	—
	5	196	7.97	2.46	110	38	6.62	.94	2.96	65	—	—

‡ Atrial pressures.
§ Atrial sampling throughout; PA pressures recorded during comparable study.

TABLE I—*Continued*

Subject	Minute of study*	O_2 Consum. (*ml./min./m.²*) N.T.P.	A-V diff. (*vols. %*) N.T.P.	C.I. (*L./min./m.²*)	Heart rate	Stroke vol. (*ml.*)	Vent. (*L./min./m.²*) N.T.P.	R.Q.	% O_2 extn.	Pressure (*mm. Hg*) Mean P.A.	B.A. (S)	B.A. (D)
C13	Rest	137	5.93	2.31	80	55	4.43	.89	3.09	55	118	76
	Ex. 1	188	6.82	2.76	91	58	6.41	.90	2.94	—	150	95
SA = 1.90 m.²	2	214	9.87	2.16	94	44	8.51	.88	2.51	—	160	96
RC = 17.50	3	254	11.31	2.24	91	47	9.34	.92	2.71	—	150	92
EC = 17.95	4	261	11.67	2.24	100	43	9.64	.94	2.71	78	144	92
	5	243	11.49	2.12	115	35	9.34	.98	2.51	78	144	90
	Ry. 2	163	8.26	1.97	80	47	6.25	.98	2.60	68	120	74
	5	129	6.82	1.89	74	49	4.82	.97	2.67	60	120	88
C14	Rest	128	5.01	2.56	80	53	3.69	.94	3.48	40	124	76
	Ex. 1	147	7.47	1.97	101	32	3.49	.88	4.22	60	140	90
SA = 1.65 m.²	2	263	9.50	2.77	110	42	6.02	.81	4.37	70	140	90
RC = 19.60	3	273	10.50	2.60	105	41	6.54	.85	4.17	70	140	90
EC = 20.20	4	261	11.10	2.35	110	35	6.80	.90	3.84	82	140	90
	5	263	11.71	2.25‖	133	28	7.22	—	—	75	—	—
	Ry. 3	205	6.87	2.99	90	55	5.76	.98	3.57	55	—	—
	5	131	6.67	1.96	90	36	3.49	.92	3.75	45	—	—
C15	Rest	176	7.14	2.47	97	42	4.89	.91	3.60	68	104	72
	Ex. 1	216	9.11	2.37	128	30	6.31	.90	3.42	—	108	74
SA = 1.64 m.²	2	273	11.63	2.34	148	26	9.00	.97	3.03	—	120	78
RC = 18.64	3	299	12.17	2.46	160	25	10.37	1.03	2.88	—	120	80
EC = 19.14	4	273	12.00	2.27	168	22	10.71	1.14	2.55	85	120	80
	5	268	12.38	2.16	160	22	11.23	1.15	2.39	—	124	80
	Ry. 1	227	12.13	1.88	152	20	8.81	1.12	2.58	—	122	80
	2	201	9.41	2.13	141	25	7.60	1.14	2.64	—	124	80
	3	182	8.07	2.26	111	33	6.62	1.11	2.75	—	124	76
	4	173	7.73	2.24	121	30	6.13	1.09	2.82	—	124	76
	5	179	8.29	2.16	147	24	5.70	.93	3.14	70	118	82
C16	Rest	135	9.68	1.39	68	36	4.30	.96	3.13	55	120	70
	Ex. 1	157	10.25	1.53	117	23	6.07	1.00	2.58	—	120	70
SA = 1.76 m.²	2	184	13.55	1.35	140	17	7.69	.94	2.39	—	120	70
RC = 20.34	3	232	15.96	1.45	140	18	9.57	.99	2.43	—	130	70
EC = 20.90	4	245	17.43	1.40	140	18	11.28	1.09	2.17	64	120	70
	5	299	17.96	1.66	140	21	12.14	1.08	2.46	—	120	70
	Ry. 1	295	17.22	1.71	123	24	10.97	1.06	2.69	—	115	70
	2	235	15.92	1.48	103	25	10.08	1.13	2.33	—	120	70
	5	151	12.39	1.22	90	24	6.30	1.10	2.40	30‡	130	70

‖ O_2 extraction assumed from mean of mins. 2, 3, and 4.

Exercise continued for five minutes at a steady rate. During this period, and for five minutes thereafter, two to four 3 ml. samples of mixed venous blood were drawn through the catheter each minute, the greater number of samples being taken during early exercise and early recovery. In the short intervals between sampling, the collecting arm of the manifold was allowed to drip slowly to ensure that the initial blood drawn from the catheter was representative of the inflowing mixed venous blood. Arterial blood samples were taken every one or two minutes, but if arterial blood desaturation was suspected, sampling was more frequent. All blood samples were drawn at as steady a rate as possible and the times of each sampling period noted. Meanwhile the expired air was collected in the Tissot spirometer and expired gas sampled throughout each minute. If the spirometer bell became nearly full during the study, the expired air was diverted to a Douglas bag while the bell was emptied.

Pulmonary artery pressures were recorded at rest and as often during exercise and recovery as was feasible. This could be done rapidly by turning the manifold connection attached to the catheter from the sampling arm to the electromanometer. Arterial pressures were also recorded while blood sampling was not being carried out and although some of these records were damped, the majority were satisfactory and the pulse rate and rhythm were followed throughout the exercise and recovery

One member of the team was made specifically responsible for constant observation of the patient's condition and was empowered to stop the exercise at once if he thought fit. The patient was also instructed that, although he would probably be moderately breathless, he should stop at once, without hesitation, if he felt at all unwell.

Studies were performed on three main groups of patients who exercised at rates of work and oxygen consumption corresponding very approximately to walking on the flat at 3 m.p.h., 2 m.p.h., and 1 m.p.h.

TABLE II

Degree of dyspnoea and ventilation, M.V.B. saturation, cardiac output and vascular pressure changes

Subject	Degree of dyspnoea	Ex. vent. 5th min. (*L./min./m.²**)	M.B.C. (*L./min./m.²**)	Vent./M.B.C. ×100	Ex. level M.V.B. 5th min. (% *sat.*)	Mean pressures (*mm. Hg*) "Pulmonary capillary"	Resting P.A.	Ex. P.A.	Cardiac index (*L./min./m.²*) Resting	5th min. ex.
C1	none	9.1	48.1	15	55	22	26	30	4.21	6.17
C2	severe	15.8	29.8	53	35	26	30	66	3.44	7.99
C3	slight	16.8	54.3	31	18	28	35	65	2.12	3.49
C4	moderate	20.1	36.3	55	37	30	34	65	2.99	4.77
C5	slight	20.9	58.8	36	22	30	40	55	2.93	4.13
C6	none	12.2	37.8	37	31	26	32	66	2.59	3.28
C7	moderate	22.8	32.0	71	31	36	65	85	3.61	4.33
C8	moderate	10.5	37.2	28	29	38	48	—	2.83	2.77
C9	severe	21.1	35.2	61	21	33	50	83	3.02	3.30
C10	severe	25.6†	112.1	23	7†	40	62	80†	3.86	4.29
C11	moderate	13.7	25.3	54	37	38	55	75	2.84	3.32
C12	moderate	13.4	25.1	54	33	48	60	80	2.61	3.01
C13	slight	9.3	42.1	22	30	45	60	78	2.31	2.12
C14	moderate	7.2	22.3	32	37	26	40	82	2.56	2.25
C15	none	11.2	40.1	28	31	30	68	85	2.47	2.16
C16	moderate	12.1	39.8	26	9	48	55	64	1.39	1.66

* N.T.P.
† 4th min. values.

RESULTS

The results obtained are shown in Tables I and II and Figures 2, 3, and 5–9. In each of these figures the results in one or two normal subjects at the appropriate level of exercise are also shown. In Figure 2, illustrating the changes in mixed venous and arterial blood, each point plotted is the mean of two analyses of one sample of blood. Since blood samples were drawn at a steady rate it was considered justifiable to plot the percentage saturation of the sample at the mid-time of each collection. Abrupt changes in mixed venous blood could be plotted during rapidly changing states. It was possible from such records to calculate the mean (in time) arterio-venous oxygen content difference during each minute with considerable precision in the most variable states and, since the oxygen uptake during each minute was known, the cardiac output during that minute could be calculated. Transient differences in ventricular output cannot be appreciated by this method.

Sampling errors

Before discussing the findings obtained it is necessary to consider the possible errors that may have resulted from the use of the mean A–V difference (in time) and the oxygen uptake as determined by spirometry, during changing states, particularly in early exercise.

By plotting the percentage saturation of each steadily drawn mixed venous blood sample (10 seconds) at the mid-time of collection a curve will be obtained that represents the changing percentage saturation of the blood flowing through the pulmonary artery in relation to time. However the use of a mean A–V difference, derived from such a curve obtained with timed samples, in the Fick equation will only give an accurate calculated flow in changing states if *either* the flow *or* the A–V difference is constant over the minute period being studied (5). When both the flow and A–V difference are changing during the experimental period then the mixed venous blood saturation should be determined as each unit volume passes the point of sampling. The mean of such a series of observations would give a true mean A–V difference of the blood that has passed into the lungs which could be confidently applied to the Fick equation. Such a procedure is quite beyond present methods and knowledge; indeed, the problems being studied would have long been solved if pulmonary blood flow could be measured instantaneously and accurately.

The repeated demonstration of a steady mixed venous blood saturation and constant or nearly constant A–V difference after a period of exercise allowed the Fick principle to be applied with great confidence. It was shown, in seven of the sixteen patients studied, that no increase of cardiac output

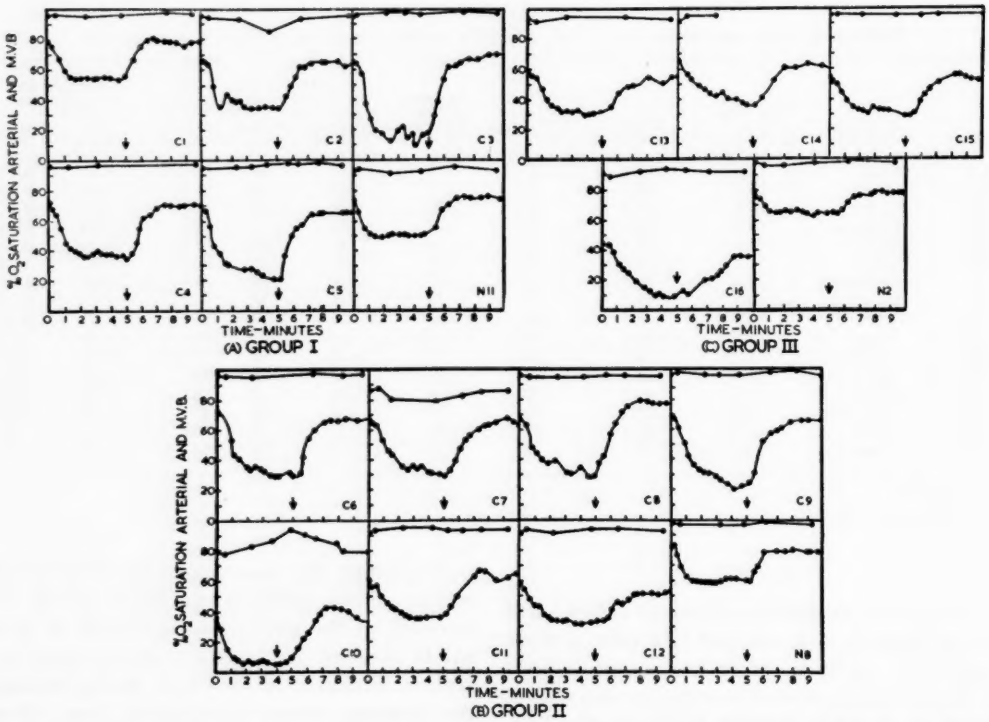

FIG. 2. ARTERIAL AND MIXED VENOUS BLOOD SATURATION DURING EXERCISE AND RECOVERY IN RHEUMATIC HEART DISEASE

C. Rheumatic heart disease. N, normal subject. Exercise commences at time 0, when resting values are plotted, and ends at signal. Max. oxygen uptake (ml./min./m². N.T.P.) Group I, 550 to 750. Group II, 360 to 490. Group III, 260 to 300.

occurred as judged by the resting steady state and exercise steady state determinations. It was considered, in these circumstances, most improbable that any increase in flow occurred during the unsteady state in early exercise and therefore, although the A–V difference was changing rapidly, the Fick principle could still be applied to timed samples without error. The fact that the cardiac output in early minutes of exercise, as calculated from timed samples, was equal to or very close to the values obtained in the resting and exercise steady states lent strong support to these theoretical considerations.

In six other patients the rise in cardiac output was so slight that, again, flow considerations were not important.

In Group I three patients showed a normal or almost normal rise of cardiac output on exercise, as judged by resting steady state and exercise steady state determinations, and it is of interest to estimate the order of error of calculated flows that resulted from the use of a mean A–V difference derived from timed samples in the first minute of exercise. An analysis, as suggested by Visscher and Johnson (5), of the errors in estimation of flow caused by the use of mean (time sampling) A–V difference over a period has been carried out on the data of the first minute of exercise in these three patients. As Visscher pointed out, the order of error of calculated flow with changing flow and A–V difference is a function of the ratios of flow and A–V difference during the two half-periods of the total period under consideration. In these three subjects the ratios of the half-minute mean A–V differences (time samples) could, with the present technique, be deter-

mined with some precision and they were as follows: patient C1, 1 : 1.48, patient C2, 1 : 1.29, patient C4, 1 : 1.44. The ratio of flow in the two half minutes was not known but a reasonable procedure to define the limit of error was to consider this ratio to be that of the resting cardiac output to the cardiac output in the second minute of exercise. In the three patients this ratio was as follows: C1, 1 : 1.40; C2, 1 : 1.51; and C4, 1 : 1.57. The calculated percentage error in flow determination resulting from the use of the minute mean A–V difference (in time) under these conditions was + 3 per cent, + 3 per cent, and + 4 per cent, respectively. These errors, although systematic, are of a lesser order than the differences between immediately repeated observations of the cardiac output in the resting steady state under the most rigidly controlled experimental conditions (4). Thus, even in these patients with a normal or nearly normal response to exercise, the error is apparently unimportant. While it is agreed that such errors must be carefully assessed it is also felt that the possible changes in flow and A–V difference and the sampling errors therefrom have been greatly overestimated.

Next the problem of the accuracy of the oxygen uptake determinations during rapidly changing states must be considered. As has been mentioned, the steady sampling of expired gas throughout each minute has again laid the investigators open to the errors that occur with "time sampling" when there are changes in both gas flow and oxygen extraction. The reasonably satisfactory checks shown in Figure 1 between what is in essence "time sampling" and "flow sampling" in this series is due to the almost constant oxygen extraction at all times in these patients with heart disease. Under such conditions, as pointed out

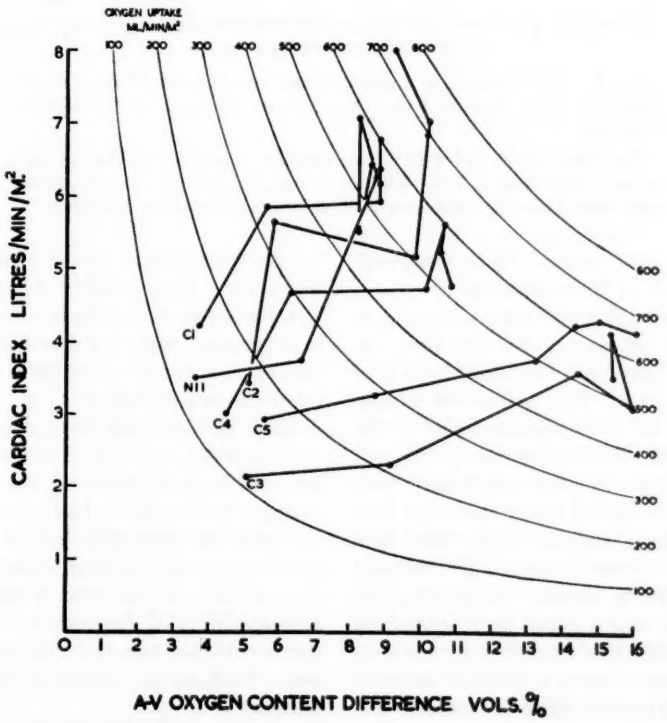

FIG. 3. A–V DIFFERENCE, CARDIAC INDEX AND OXYGEN UPTAKE AT REST AND DURING EACH MINUTE OF FIVE MINUTES EXERCISE GROUP I (C1–5, O_2 UPTAKE, 550 TO 750 ML./MIN./M².)

N, normal subject. All gas vols. at N.T.P.

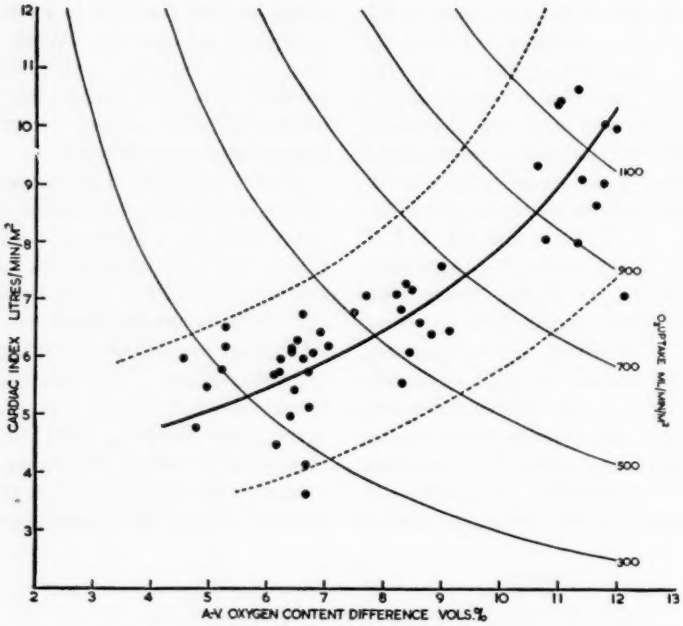

FIG. 4. A–V DIFFERENCE, CARDIAC INDEX AND OXYGEN UPTAKE DURING EXERCISE, AFTER STEADY STATE HAD BEEN ACHIEVED IN 16 NORMAL SUBJECTS

The third, fourth and fifth minute exercise results are plotted in each subject. Gas vols. N.T.P. Regression line and 95 per cent confidence limits shown are calculated from A–V difference/O_2 uptake relationships.

in relation to the Fick method, "time sampling" is almost as accurate as "flow sampling."

Even if the figures obtained were very close to the actual amount of oxygen that had been extracted from the expired air, there may have been further sources of error. The "spirometer oxygen uptake" may not be truly representative of the oxygen uptake by the blood in its passage through the lungs. A change in mean respiratory level of the chest and volume of air in the lungs during the early stages of exercise would cause such an error, as the volume of expired air collected would be appropriately altered. If the mean respiratory level and lung volume were reduced by a certain amount then this volume of gas entering the spirometer would cause a positive error of oxygen uptake estimation since its oxygen tension would not have reached these reduced levels by transfer of oxygen to blood during the experimental period. In the same way retention of inspired gas that had lost oxygen to the blood during the experimental period would cause a negative error. This possible change in respiratory level during exercise is most difficult to assess and is at present mainly a theoretical source of error. However, scrutiny of the data shows a low and relatively unchanging level of oxygen extraction in these patients with heart disease, even during early exercise, and it is of the order of 2.5 to 4.0 per cent in most cases. If the respiratory level changed by as much as half a litre then the resultant error in measuring "blood oxygen uptake" from "spirometer oxygen uptake" would only be 12 to 20 ml. and the error in cardiac output estimation with A–V differences of 5 to 10 vols. per cent would therefore be of the order of .02 L. per min. Such errors cannot be considered of importance.

Changes in the alveolar oxygen concentrations throughout the lungs in early exercise are another cause of difference between "spirometer" and "blood" oxygen uptakes. Approximate calcula-

tions of the changes of alveolar oxygen percentages between the resting state and the first minute of exercise, using the expired oxygen and carbon dioxide concentrations and assuming an effective tidal air of 75 per cent, gave the following results. In seven subjects the alveolar oxygen changed by less than 0.25 per cent, in five by 0.25 per cent to 0.5 per cent, in three by changes of between 0.7 per cent and 0.8 per cent and in one by 1.33 per cent. In ten cases the alveolar oxygen percentage was raised, in five lowered, and in one unchanged. If the lungs are assumed to have held two and a half liters of gas in the mid respiratory position then the resultant difference between "spirometer" and "blood" oxygen uptake in the first minute of exercise varied from 5 ml. (0.2 per cent change) to 25 ml. (1.0 per cent change) and in the majority of cases was below 10 ml. Again such errors will be of no importance with present techniques.

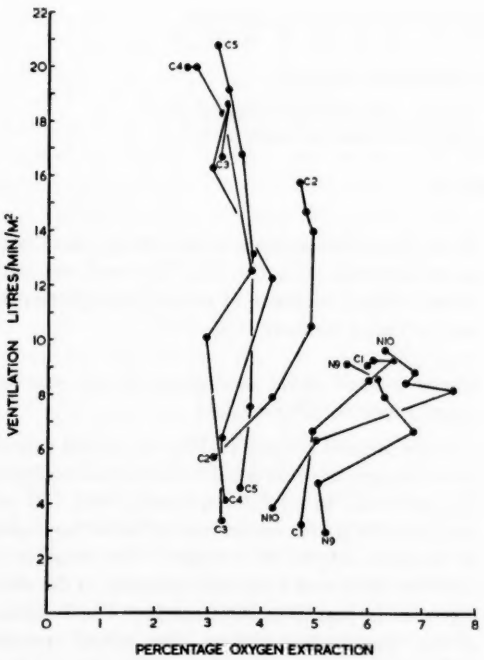

FIG. 5. VENTILATION AND PERCENTAGE OXYGEN EXTRACTION AT REST AND DURING EACH MINUTE OF FIVE MINUTES EXERCISE IN GROUP I (C1–5, O_2 UPTAKE, 550 TO 750 ML./MIN./M².)

N, normal subject. All gas vols. at N.T.P.

It has been suggested that imbalance between oxygen uptake by the blood in the lungs and body oxygen usage may cause inaccuracies when the Fick method is employed in changing states. It is difficult to see how such considerations affect the Fick principle when it is being applied only to the lungs and the blood flowing through them. Again, lag in changes in expired air in relation to blood oxygen uptake is unlikely to be very marked in view of the increased ventilation and respiratory rates of these subjects.

In summary, it can be stated that the errors resulting from time-sampling of the mixed venous blood during the changing state are of little importance in these studies of patients with heart disease. It has been shown that even with a normal response of cardiac output the errors caused by time sampling of mixed venous blood during early exercise are far less than the differences reported between repeated resting cardiac output estimations under ideal experimental conditions. A number of factors have combined to reduce errors in the estimation of the true oxygen uptake by the blood in the lungs during early exercise. However, in normal subjects with marked increase in oxygen extraction and fall in alveolar oxygen concentration during early exercise the difference between "spirometer" and "blood" oxygen uptake may cause significant errors of cardiac output estimation.

The steady state

There are obvious difficulties in defining a steady state when dealing with biological phenomena particularly when such a labile function as circulation is being considered. If the definition demanded that absolutely no change occurred in any of the parameters being measured, then a completely steady state would be a great rarity and in order for it to be demonstrated then either the techniques of sampling and analysis would have to be perfect or their errors absolutely constant. In this work the authors were specifically interested in the constancy or otherwise of the measurements that are employed to estimate the cardiac output by the Fick principle and it was considered that a steady state had been achieved when there was no important change or persistent trend of the oxygen uptake, arteriovenous difference or derived

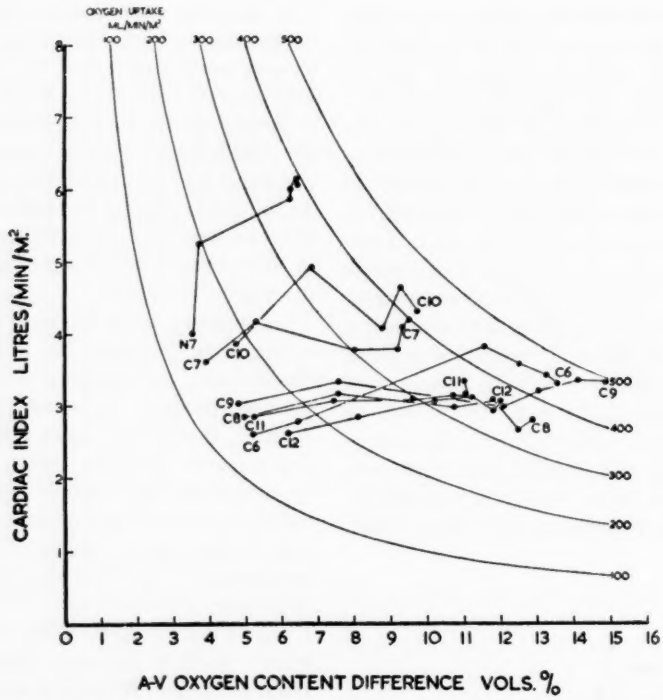

FIG. 6. A–V DIFFERENCE, CARDIAC INDEX AND OXYGEN UPTAKE AT REST AND DURING EACH MINUTE OF FIVE MINUTES EXERCISE GROUP II (C6–C12, O_2 UPTAKE 360 TO 490 ML./MIN./M².)

N, normal subject. All gas vols. at N.T.P.

cardiac output. This definition is inevitably open to slightly varying interpretations but this is not important to the main findings. In discussing the results the term steady state is therefore employed only in this special sense and its use does not imply constancy of ventilation, of heart rate, or of any measurements other than the oxygen uptake and A–V difference.

DISCUSSION OF RESULTS

Since the patients were divided into three groups, according to the level of work they could sustain for five minutes the main findings in each group will be discussed separately.

Group One

These patients were capable of considerable exertion and maintained a degree of exercise for five minutes that involved a maximum oxygen uptake of 550 to 750 ml. per min. per m² (N.T.P.) There were five patients in this group, four with mitral stenosis (C1, C2, C4, C5) and one with strong clinical evidence of mitral regurgitation as well as mitral stenosis (C3).

Mixed venous blood saturation during exercise and recovery (Figure 2A)

In the normal subject (N11), the mixed venous blood oxygen saturation fell from the resting figure (73 per cent) to a relatively steady level (50 per cent) in a little over one minute after the beginning of the same degree of exercise. On cessation of exercise there was a prompt recovery to the resting level in just over 90 seconds. The behavior of the symptomless patient with mitral stenosis (C1) was similar in all respects.

The more disabled patients all attained an abnormally low mixed venous blood oxygen saturation with this level of exercise, varying from 38 per cent to 17 per cent (normal 47 per cent to 54

per cent), (2) and although the fall in mixed venous blood saturation was precipitous, there was an appreciable delay in the achievement of a relatively even level of mixed venous blood saturation, particularly in C5 (M.S. Grade 3a).[2] On cessation of exercise, the mixed venous blood oxygen saturation returned to the resting level in the same time and therefore at an even greater speed than in normal subjects.

Cardiac output, A–V difference and oxygen uptake during exercise

In Figure 3 the derived cardiac index has been plotted against the A–V difference. As the oxygen consumption per square metre is the multiple of these two values a third series of curved coordinates showing various levels of oxygen consumption can also be constructed. The resting values of cardiac index and A–V difference in each case are first plotted, and the appropriate values obtained in the first to the fifth minutes of exercise are also plotted and joined by straight lines. Thus the behaviour of the cardiac index, A–V difference and oxygen consumption can be followed from minute to minute during five minutes exercise in each subject. For the purpose of comparison, Figure 4 shows the cardiac output plotted against the A–V difference after a steady state had been achieved during exercise in a study of 16 normal subjects by the authors. The regression line and 95 per cent confidence limits are also shown. All figures described as abnormal in this paper are outside these limits.

In the normal subject N11 (Figure 3) the resting cardiac index was 3.5 L. per min per m². ; A–V difference 3.6 ml. per 100 ml. and oxygen uptake 125 ml. per min. per m². During the first minute of exercise the cardiac index increased to 3.7 L. per min. per m². the A–V difference to 6.6 ml. per 100 ml., and the oxygen uptake to 250 ml. per min. per m². The cardiac index, A–V difference, and oxygen uptake were relatively steady in the second, third, fourth, and fifth minutes of exercise, with a cardiac index of the order of 6.3 L. per min., A–V difference of 8.4 ml. per 100 ml., and oxygen uptake of 535 ml. per min. per m². In the study of normal subjects, of which N11 is typical, it was demonstrated that a steady state, as previously defined, was reached after one minute in all subjects exercising at levels comparable to those used in the present studies.

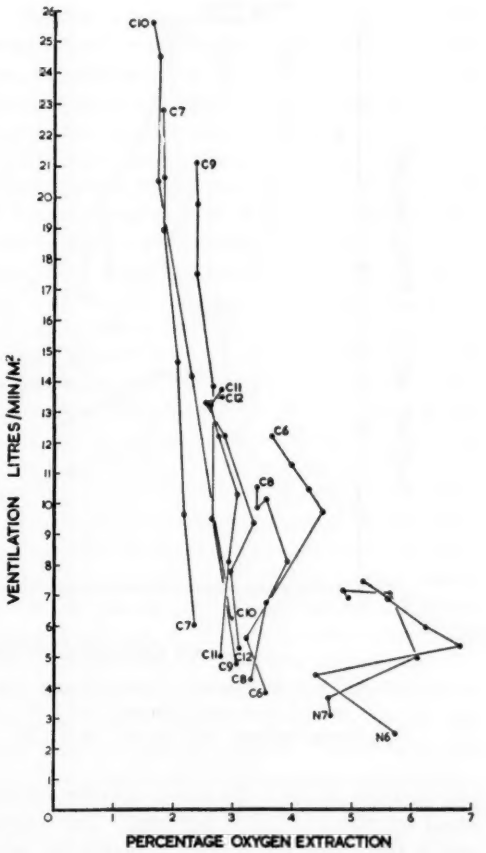

FIG. 7. VENTILATION AND PERCENTAGE OXYGEN EXTRACTION AT REST AND DURING EACH MINUTE OF FIVE MINUTES EXERCISE IN GROUP II (C6–C12, O_2 UPTAKE 360 TO 490 ML./MIN./M².)

N, normal subject. All gas vols. at N.T.P.

The patient C1 with symptomless mitral stenosis, behaved in a similar manner to the normal subject, achieving a steady state in the same time. C2 (M.S., Grade 2) showed what appeared to be a normal response of cardiac output and resultant A–V difference and came to an apparently steady state in the third and fourth minutes. In the fifth minute he developed frank pulmonary edema (the

[2] Grading of disability according to American Heart Association. See footnote to Appendix concerning subdivision of Grade 3.

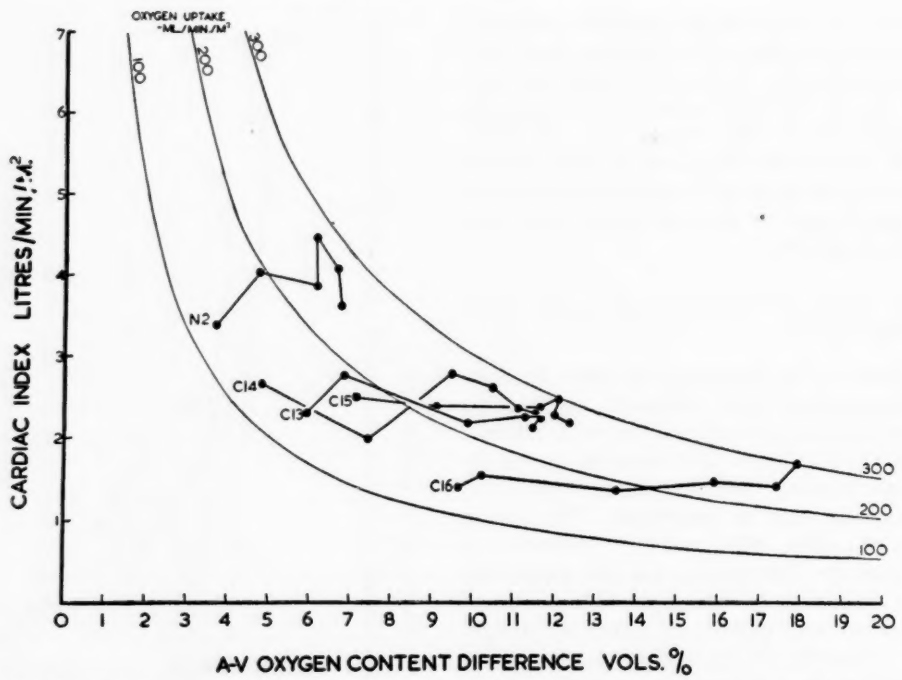

FIG. 8. A–V DIFFERENCE, CARDIAC INDEX AND OXYGEN UPTAKE AT REST AND DURING EACH MINUTE OF FIVE MINUTES EXERCISE, GROUP III (C13–C16, O_2 UPTAKE 260 TO 300 ML./MIN./M².)
N, normal subject. All gas vols. at N.T.P.

only instance of this in the series) and the arterial blood saturation fell to 86 per cent. The cardiac output then increased further, although the oxygen uptake remained almost constant. The signs and symptoms of pulmonary edema cleared rapidly with the cessation of exercise and the arterial blood saturation returned to its previous level (96 per cent) after five minutes recovery. C3 (M.S. and R., Grade 2) had a low resting cardiac index which rose but little on exercise with a resulting abnormal increase of A–V difference. Despite the abnormally low cardiac output and greatly increased oxygen extraction this patient achieved a relatively steady state *after* the second minute of exercise. C4 (M.S. Grade 3a) showed a slightly impaired rise of cardiac output and abnormal A–V difference (see Figure 4) and C5 a very slight increase of cardiac output and highly abnormal increase of A–V difference. C4 achieved a steady state *after* the first minute of exercise but C5, who showed but little increase of cardiac output, took two minutes to reach only a relatively steady state and was still increasing the A–V difference up to the end of exercise.

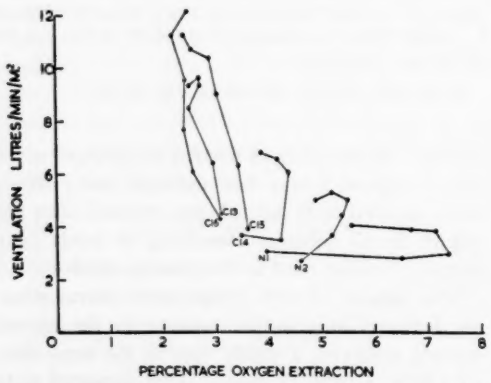

FIG. 9. VENTILATION AND PERCENTAGE OXYGEN EXTRACTION AT REST AND DURING EACH MINUTE OF FIVE MINUTES EXERCISE IN GROUP III (C13–C16, O_2 UPTAKE 260 TO 300 ML./MIN./M².)
N, normal subject. All gas vols. at N.T.P.

Ventilation and ventilatory equivalent during exercise

In Figure 5 the ventilation is plotted against the percentage oxygen extraction. The normal subjects N9, and N10 showed a considerable increase of oxygen extraction in the early minutes of exercise and, by the fifth minute, the extraction was returning towards the resting level and the ventilation was almost contant. The behavior of the ventilation and oxygen extraction in the patient with symptomless mitral stenosis (C1) was similar to that of the normal subjects. The remaining patients showed, on exercise, a marked ventilatory response with little change in oxygen extraction except in the case of C2 (Grade 2). Patients C2, and C5 were still increasing their ventilation considerably at the end of the five minutes exercise.

Group Two

The patients in this group were considerably disabled and were only capable of five minutes exercise involving a maximum oxygen uptake of 360 to 490 ml. per min. per m². (N.T.P.) There were seven subjects (see Appendix); four with mitral stenosis (C7, C8, C9, C10), one with mitral stenosis and regurgitation (C11), one with mitral stenosis and aortic stenosis (C12), and one with signs of predominant and severe mitral regurgitation (C6).

Mixed venous blood saturation on exercise and recovery

The behavior of the mixed venous blood saturation in these patients and in a normal subject (N8), performing the same degree of exercise is shown in Figure 2B. The percentage saturation of the mixed venous blood fell rapidly to an abnormally low level for this degree of exercise (28 per cent to 40 per cent;[3] normal, 55 per cent to 65 per cent). There was again some delay in achieving a relatively steady level of mixed venous blood saturation but all subjects had achieved this in two to three minutes with the exception of C9 (M.S., Grade 3b). There was an abrupt return to resting levels within 90 to 120 seconds of cessation of exercise, with only one exception (C10).

[3] C10 excepted.

C10 not only had mitral stenosis but was also anemic (Hb. 8.25 Gm. per 100 ml.), and consequently although the resting cardiac output was normal, the mixed venous blood percentage saturation was at the low level of 37 per cent. On exercising the combination of anemia, and the inability to raise the cardiac output normally, caused a fall of mixed venous blood percentage saturation to the astonishing figure of 6 per cent. (Figure 2B). This patient had to stop exercising after four minutes owing to very severe dyspnoea and there was a considerable delay in recovery of mixed venous blood oxygenation to the resting level. The mixed venous blood saturation finally rose above the resting level, and fell again to that level. This phenomenon was almost certainly due to the fact that this patient was suffering from arterial blood desaturation at rest (79.6 per cent), achieved relatively normal saturation at the end of exercise and during early recovery (92.7 per cent), and then returned to the previous arterial saturation (78.7 per cent). This odd behavior of arterial oxygenation on exercise has been observed in other anemic subjects without heart disease.

In the case of C12, the catheter tip would not remain in the pulmonary artery and atrial samples were collected throughout. The return of the mixed venous blood saturation to a level considerably below the resting level may have been due to the vagaries of atrial sampling and the figures in this case must be viewed with some reserve. Similarly, in the study of C8 (M.S., Grade 3b) the tip of the catheter slipped back into the right atrium during exercise. The mixed venous blood saturation rose on recovery to well above the resting level and this rise was sustained to the end of the study. If these figures were accepted then it would appear that, although this patient was unable to raise the cardiac output on exercise, there was a significant increase during recovery. Again it is possible that this unusual finding is due to the uncertainties of atrial sampling.

Cardiac output, A-V difference, and oxygen uptake during exercise (Figure 6)

In a normal subject (N7), performing the same degree of work, the increase of oxygen uptake was effected by an approximately 50 per cent increase in cardiac output and a doubling of the A–V dif-

ference. The rapid achievement of the steady state, as measured by these values, *after* the first minute is also shown.

The behavior of the patients with heart disease contrasted with the normal subjects even more strikingly than in the previous group. The majority showed no ability to increase the cardiac output as oxygen uptake increased, the A–V difference increasing almost proportionately to the oxygen uptake. This was best shown in cases C8 (M.S., Grade 3b), C9 (M.S., Grade 3b), C11 (M.S. and R., Grade 3b) and C12 (M. and A.S., Grade 4), all of whom had low resting cardiac outputs. Despite the fixed cardiac output and abnormally increased A–V difference, C8, C11, and C12 reached a steady state *after* two minutes but C9 (M.S., Grade 3b) still showed an increase of oxygen uptake and A–V difference up to the fifth minute of exercise. C6 (M.R.), a patient with Grade 2 disability and the only member of the group to suffer no dyspnoea at this level of exercise, had a resting cardiac index as low as the severely disabled patient (C12) with severe mitral stenosis and aortic stenosis. In the first two minutes of exercise the cardiac output increased by 50 per cent but during the third, fourth, and fifth minutes, although the oxygen uptake remained constant, there was a slight but steady fall of cardiac output and increase of A–V difference. In patients C7 (M.S., Grade 3b) and C10 (M.S., Grade 3b) the cardiac output was normal at rest and showed a transient rise on exercise, which was not sustained, the A–V difference increasing abnormally with increase of oxygen uptake. Both subjects achieved a steady state *after* the second minute of exercise.

Ventilation and ventilatory equivalent during exercise

These patients showed a higher resting ventilation and lower oxygen extraction than normal subjects. In this small group there is quite a striking correlation between the degree of disability and the elevation of the resting ventilation and the ventilatory response to exercise. Those with the highest resting ventilation and lowest oxygen extraction showed the greatest ventilatory response (Figure 7). The ventilatory response of two normal subjects (N6 and N7) to the same degree of exercise is also shown.

Four patients C6, C7, C9, and C10 showed an increasing ventilation throughout the exercise period. Patients C8, C11, and C12 reached a steady level of ventilation and oxygen extraction in the last two or three minutes of exercise. The oxygen extraction was almost constant even during early exercise, except in the relatively fit patient C6 (Grade 2).

Group Three

The four patients in this group were all severely disabled, (Grade 3b or 4). Two of the patients had mitral stenosis (C14, C15), one had mitral stenosis with gross regurgitation (C13), and one had severe mitral and aortic stenosis (C16).

Mixed venous blood percentage saturation during exercise and recovery (Figure 2C)

During this very slight exertion, which must be paralleled not infrequently in everyday life even under the most restricted conditions, the mixed venous blood oxygenation in these patients reached very low levels. Patient C14 (M.S., Grade 3b) and patient C16 (M. and A.S., Grade 4) showed great delay in reaching a steady mixed venous blood saturation and, although the levels of percentage saturation reached by these two patients were very different (36 and 8 per cent), they appear to approach a constant level at the end of exercise. When the exercise ceased the oxygen saturation of the mixed venous blood did not regain its resting level in C13 (M.R., Grade 3b) or C15 (M.S., Grade 4) for three minutes and the recovery was not complete in five minutes in patient C16 (M. and A.S., Grade 4). There was no marked change in arterial blood saturation on exercise in this group.

Cardiac output A–V difference and oxygen uptake during exercise (Figure 8)

The behavior of a normal subject N2 at this level of exercise indicates that although there is a relatively steady state *after* one minute's exercise, there is still considerable "seeking." This may be due to the fact that normal subjects find this very light leg work rather unnatural and difficult to maintain at a steady level. Some normal subjects, who had a slightly raised resting cardiac output and small A–V difference due to some de-

gree of nervous tension, did little more on exercising at this level than increase their A–V difference (2).

All four patients had resting cardiac indices below 3 L. per min. per m². and the patient with aortic and mitral stenosis (C16) had a resting cardiac index of 1.39 L. per min. per m². On exercise, none of these patients showed any increase of cardiac output and in patients C13 (M.R.), C14 (M.S.), and C15 ,M.S.) the final cardiac output was slightly less than in the resting state. In all cases, therefore, the A–V difference increased almost proportionately to the increase in oxygen uptake.

As judged by these measurements C15 (M.S., Grade 4) achieved a remarkably steady state *after* one minute and C13 (M.R., Grade 3b) *after* two minutes. C14 (M.S., Grade 3b) did not achieve such a steady state and although the oxygen uptake was almost constant after the first minute of exercise, the cardiac output was decreasing and the A–V difference increasing in the fifth minute. C16 (M. and A.S., Grade 4) with the very low cardiac output, showed an abnormal, and increasing A–V difference from the first to the fifth minute.

Ventilation and ventilatory equivalent (Figure 9)

In three of these patients the exercise ventilation was greatly increased as compared with the normal subjects. One of these patients, C13, had reached a steady level of ventilation after the second minute but in the others it was still increasing in the fifth minute of exercise, with decreasing oxygen extraction. Patient C14 (M.S., Grade 3b) had only moderately increased ventilation and showed increased oxygen extraction during the first three minutes of exercise, behavior more like that of a normal subject.

Behavior of A–V Difference and Cardiac Output during Recovery in All Groups

In the early stages of this study the oxygen uptake was only determined in the second and fifth minute of recovery, but minute to minute recovery studies were made in nine patients. The behavior of normal subjects during recovery after five minutes exercise at comparable levels was remarkably constant (2). Immediately after exercise ceased the cardiac output, A–V difference and oxygen uptake fell rapidly and, by the second minute of recovery, these values were very close to, if not at, the pre-exercise values, which they invariably achieved in the third minute of recovery.

In all of the cardiac patients the mixed venous blood saturation had returned to pre-exercise levels by the second minute of recovery with the exception of patients C12 and C16, both of whom had aortic stenosis in addition to mitral stenosis. Despite this the A–V difference remained increased in many subjects up to the fifth minute of recovery, partly because the blood oxygen capacity was assumed to remain at its increased exercise level during this time and partly because ventilation remained considerably increased thus causing a slight rise of the arterial blood saturation during early recovery.

In those patients in whom the cardiac output rose considerably on exercise the rate of return to the resting output level was normal in patients C1 (Grade 1) and C4 (Grade 3a) and probably slightly delayed in patient C2 (Grade 2). In the six patients with only a slight rise of cardiac output on exercise, the output returned to its pre-exercise level by the third minute of exercise in only two patients, C5 (Grade 3a) and C6 (Grade 2), neither of whom was very disabled. In patient C3 (Grade 2) who was not very disabled but had recently been in failure, the return of the cardiac output to resting levels was not complete by the fifth minute of recovery. A secondary rise of cardiac output occurred during recovery in patients C7, C8, and C10 (all Grade 3b) and this delayed the return to resting levels until at least the fifth minute of recovery. No explanation can be given for this secondary rise but in patient C8 the finding may have been due to the errors of atrial sampling. In the remainder of the patients (all Grades 3b and 4) the cardiac output remained constant throughout exercise and recovery but in all cases the A–V difference remained elevated until the fifth minute of recovery, this being most marked in patient C16 in whom it was still almost a third above the resting value in the fifth minute of recovery. In all patients the ventilation was still increased in the fifth minute of recovery and in many, especially in those performing the highest level of exercise, this increase was considerable.

Pulse Rate and Stroke Volume during Exercise and Recovery

The pulse rate increased during exercise in all subjects. Eight of the patients achieved a steady rate after the second minute and three after the third minute of exercise. Rather surprisingly, three patients with auricular fibrillation and "fixed" cardiac outputs achieved a constant pulse rate after the first minute of exercise. Two very disabled patients increased their pulse rate throughout although one achieved a very steady oxygen uptake, cardiac output and A–V difference after two minutes (C13). In some patients the pulse rate was steady before the cardiac output and A–V difference stabilized and in other patients after. In three instances a constant pulse rate was achieved although the patients never reached a steady state as judged by oxygen uptake, cardiac output and A–V difference. These findings show that constancy of pulse rate is not a reliable criterion of steadiness of the A–V difference and cardiac output.

The heart rate was slow to return to resting levels after exercise and in many subjects had not returned to pre-exercise levels by the fifth minute of recovery, especially in the group performing the heaviest exercise.

The stroke volume fell during exercise in all subjects except C1 and C2, although C10 and C13 showed a small transient increase during the first minute. A steady level of stroke volume was achieved at least during the second minute of exercise and in several cases during the first minute. Although stroke volumes are given in all studies these figures are of little value in the patients with auricular fibrillation. Stroke volumes were lower in the fifth minute of recovery than before exercise in the majority of the subjects.

Respiratory Quotient during Exercise and Recovery

In all these patients the respiratory quotient (R.Q.) was almost constant in the fourth and fifth minutes of exercise and in some patients by the third minute. In the majority of patients who achieved a steady A–V difference and cardiac output, the R.Q. did not stabilize until a minute or two later. However in three patients (C5, C9, C16), who did not achieve a constant A–V difference and cardiac output, the R.Q. reached a nearly constant value in the last two or three minutes of exercise. In many instances a fall of the R.Q. occurred during the first one or two minutes of exercise but this was never marked and of a lesser order than in normal subjects under the same conditions. In those patients performing the lightest work there was little change of the R.Q. during exercise, except in C15. In all patients in all groups, with the exception of C2, the final exercising R.Q. was higher than the resting value.

During recovery, with continued high ventilatory volumes and rapid fall of oxygen uptake, nearly all subjects showed a further rise of R.Q. to well over unity, and in several cases to very high values (C10, 1.44 and C5, 1.71). Only subjects C8, C9, and C11 showed any re-establishment of a steady R.Q. during the recovery study, this being in the fourth and fifth minute of recovery, yet the majority of patients had returned to a steady near-resting cardiac output and A–V difference towards the end of five minutes recovery. These observations indicate that it is unwise to infer the degree of steadiness of the cardiac output and A–V difference from the constancy or otherwise of the respiratory quotient.

Oxygen Uptake and Oxygen Debt

The present observations demonstrate the difficulty in obtaining constancy of oxygen uptake during exercise even under carefully controlled conditions. Also, a steady oxygen uptake during exercise does not necessarily mean that a steady A–V difference and cardiac output have been achieved. Subjects C2, C6, and C14 all showed changing cardiac outputs and A–V differences although the oxygen uptake had become constant (see Figures 3, 6, and 8).

As the recovery studies were incomplete in a number of subjects and, in any case, were only continued for five minutes, useful data concerning the oxygen debt are possible in only a few instances. Normal subjects carrying out similar exercise to the patients in Group I, involved oxygen debts of about 15 per cent of the total excess oxygen consumption and this was almost completely "paid off" during the first minute of recovery. Patients C3 and C5 had oxygen debts

of the order of 22 per cent and 28 per cent of excess oxygen consumption, of which only half was paid off in the first minute and the rest more slowly.

At the level of exercise of the second group, normal subjects incurred an oxygen debt of 12 to 15 per cent of the total excess oxygen consumption, which they paid off almost completely in the first minute of recovery. Patient C6 showed a 24 per cent oxygen debt and paid off only 8 per cent in the first minute, patient C10 a 29 per cent oxygen debt of which 13 per cent was paid off in the first minute, and patient C11 a 20 per cent oxygen debt of which only 5 per cent was paid off in the first minute of recovery. All three patients had returned to the resting level of oxygen uptake by the fifth minute of recovery.

Finally, only patient C1 (asymptomatic mitral stenosis) in the first group, five of the seven in the second group, and three of the four in the third group had returned to the resting level of oxygen uptake by the fifth minute of recovery. Summarizing, it was confirmed that patients with heart disease showed an increased and prolonged oxygen debt as compared with normal subjects.

Oxygen Transfer in Lungs and Tissues

As already reported by many workers the resting arterial blood saturation is normal or nearly normal in the large majority of patients with rheumatic heart disease even in the later stages when the patient has been repeatedly in congestive failure. In the present series the arterial saturation was 94.8 per cent or above in all those capable of the highest grade of exercise (Group I, C1–5). In four of these patients the arterial saturation was maintained during exercise and rose significantly after exercise while increased ventilation was continued. The one exception in this group was the patient who developed pulmonary edema and whose arterial saturation fell from 96.6 per cent to 86 per cent returning to the original level after five minutes recovery.

In Group II (C6–C12), three patients (C6, C8, C9) had normal resting arterial saturation which behaved in the same manner on exercise as in Group I. Patients C11 and C12 who were the most disabled in this group, had a slightly lower arterial saturation 90.7 per cent and 92.0 per cent, respectively, but both improved their arterial blood saturation slightly during exercise and recovery. Patient C7 (M.S., Grade 3b) who had a resting arterial saturation of 86.9 per cent which fell to 80.2 per cent on exercise, had no history of asthma or respiratory infection and is a good example of how moderate arterial desaturation can occur in a patient with mitral stenosis whose past history is in no way exceptional. Carroll, Cohn, and Riley (6) have demonstrated disturbance of both gas and blood distribution and of the diffusing capacity of the lungs in mitral stenosis but, as yet, there appears to be no definite explanation of the mechanisms involved or of their apparently fortuitous occurrence. Patient C10, exceptional in that he was anemic as well, showed considerable arterial desaturation at rest, which disappeared on exercise, and returned during recovery.

In Group III, there was slight arterial desaturation (92.7 per cent, 91.1 per cent, 91.3 per cent) in three instances, the arterial saturation improving considerably on exercise and during recovery. One patient had normal arterial blood oxygenation throughout.

Despite the marked and long standing rise of vascular pressures throughout the lungs, the transfer of oxygen to the blood passing through them remained efficient in the majority of these patients. Normal arterial saturation was maintained even when the mixed venous blood was returning to the lungs at levels of oxygen saturation of between 6 per cent and 20 per cent. The oxygen transfer in these patients is, however, aided by several factors. The hyperventilation at rest and during exercise causes a significant rise of the alveolar oxygen tension even if there is some faulty gas distribution. This, combined with the very low oxygen tension of mixed venous blood, results in a greatly increased oxygen diffusion gradient. The low cardiac output at rest and on exercise is also associated with a relatively low blood flow velocity through the pulmonary capillaries and a corresponding increase in the time of exposure to the blood-gas interface.

Although it has long been known that cardiac and skeletal muscle is capable of functioning at low levels of oxygen tension, it has not been appreciated, at least by the authors, that striated muscle can continue to function and utilize considerable quantities of oxygen at the low levels of

oxygen tension which must have been present in the muscles of some of these patients in whom the mixed venous blood was returning to the heart less than 10 per cent saturated during exercise. The rapid rise of the oxygen saturation of the mixed venous blood after exercise would suggest that a marked "alactic acid" oxygen debt does not occur even at these low oxygen tensions, as the recovery would not be so rapid unless the local muscle oxygen demands ceased almost immediately after the end of exercise.

Dyspnoea on Exercise

Patients with heart disease have considerable difficulty in describing their symptoms while under stress. They may state that they are extremely short of breath without other symptoms, or may describe weakness, either generalized or in the exercising legs. Others report unpleasant sensations of physical inadequacy or even apprehension. A few patients will under no circumstances increase their activity over a certain level and look under obvious stress, yet will deny shortness of breath or weakness. No patient in this series with predominant mitral involvement complained of anginal pain, precordial discomfort or palpitations.

In Table II the degree of dyspnoea, as described by the patient, is shown with the maximum breathing capacity (7) and the ventilation in the last minute of exercise. The fifth minute ventilation is also expressed as a percentage of the maximum breathing capacity. Although there is a rough correlation between the percentage of the ventilatory capacity used and the degree of dyspnoea there are many individual exceptions. Patient C7 who used 71 per cent of the ventilatory capacity was only moderately dyspnoeic. Such a finding could be due to a well disguised lack of cooperation in the maximum breathing capacity determinations, but this would not explain the finding in patient C10 who was extremely dyspnoeic while using only 23 per cent of his ventilatory capacity. These limited observations are in agreement with the extensive ventilatory investigations in mitral stenosis by Frank, Cugell, Gaensler, and Ellis (8).

There was no correlation of dyspnoea with the level of mixed venous blood oxygenation achieved during exercise; C11 and C14, with a mixed venous blood saturation of 37 per cent, suffered moderate dyspnoea yet patient C3, with a mixed venous blood saturation of 17.5 per cent, was only slightly dyspnoeic. Patient C15 with a mixed venous blood saturation of 31 per cent had no shortness of breath and patient C16 suffered only moderate dyspnoea and could have continued the exercise for a longer period although the oxygen saturation of the mixed venous blood had fallen to 9.5 per cent.

Shortness of breath was studied in relation to the degree of, or lack of, increase of cardiac output on exercise. Neither dyspnoea nor ventilatory response appeared to be related to the response of the cardiac output (Table II). Similarly, no consistent relationship could be found between the ventilatory response or degree of dyspnoea and the resting pulmonary artery pressure, the resting transpulmonary pressure (P.C.P.), or the degree of rise of pulmonary artery pressure on exercise (Table II).

Regional Blood Flow Considerations

Although it was anticipated that the oxygen saturation of mixed venous blood of these patients with heart disease would fall to abnormally low levels on exercise, the extent of this fall, in some cases to levels of 6 to 10 per cent saturation, was a considerable surprise. These low figures on exercise suggested that a very marked redistribution of the flow of blood throughout the body must have occurred and it seemed likely that besides a reduction of the renal and splanchnic flow there was also a marked reduction of blood flow to the upper part of the body. The relative constancy or even elevation of the systemic blood pressure on exercise in patients who were unable to increase their cardiac output also suggested that there was widespread vaso-constriction in non-exercising zones. Work proceeds on this problem, as the technique evolved can also be used to follow the behavior of the A–V difference in various regions of the body with considerable precision during exercise and recovery or any other changing state. It has already been shown conclusively that during leg exercise in these disabled patients the oxygen content of the superior vena caval blood falls considerably and that the axillary vein blood of the non-exercising arms may

fall to even lower levels of saturation than the mixed venous blood in the pulmonary artery. When exercise ceases there is a prompt return of the percentage saturation of the axillary vein blood to the resting level. As it is unlikely that the oxygen uptake of the resting arm increases significantly during leg exercise, this demonstration of a greatly increased arteriovenous oxygen content difference in the arm is a strong indication that vasoconstriction has occurred in the limb with marked reduction of blood flow. Preliminary plethysmographic studies have confirmed that there is considerable reduction in muscle blood flow in the non-exercising arms of these patients during light exercise. The oxygen content of blood sampled from the cranial end of the jugular vein remains unchanged throughout such exercise even in gravely disabled subjects. These studies of the changes of regional A–V differences and blood flow in normal subjects and patients with heart disease during exercise are being reported in detail in another communication.

Findings in Relation to Standard Sampling Procedure

Ten of these sixteen subjects, who were exercising at different levels, but fairly near their tolerance, reached a steady state of oxygen uptake, A–V difference and cardiac output in 2 to 3 minutes. The six remaining subjects did not achieve a steady state in five minutes. One of these six patients developed pulmonary edema and need not be considered further. Three, although they had not quite reached a steady state, as judged by minute to minute studies, had actually achieved a steady level of arterial and mixed venous blood saturation by the fifth minute of exercise. In only two subjects, C5 and C14, the mixed venous blood saturation failed to reach a steady level, continuing to fall slightly up to the end of exercise. As neither patient was unduly embarrassed it is likely that they would have reached a steady state if exercise had continued. There appeared to be no important common feature in the patients who showed a considerable delay in achieving a steady state.

From consideration of these results, it would appear that "spot" samples taken in the fifth minute of exercise would have given A–V differences and cardiac outputs that would have been representative of the final steady hemodynamic state in all but two patients. Although it is impossible to be certain, it is most likely that even in these two cases the results obtained from "spot" fifth minute samples would not have been far from those of the final steady state. The occasional variation of mixed venous blood saturation around a particular level even when the patient is in an almost steady state emphasizes the need for taking at least three evenly spaced samples over the minute of study. It is also advisable to confine the collection of expired air to the period of mixed venous blood sampling.

SUMMARY

A technique has been developed whereby the behaviour of the mixed venous and arterial blood saturation can be followed accurately during rapidly changing states. It has thus been possible to determine the minute to minute changes of cardiac output in patients with rheumatic heart disease during five minutes exercise and the subsequent recovery.

Errors in cardiac output determination during early exercise due to the use of mean (in time) A–V differences and the difficulty of measuring true blood oxygen uptake are shown to be of such a small order in these studies that they are of no importance.

Particular study has been made of the rate of achievement of a steady state after the commencement of exercise. In ten out of sixteen patients a steady state of oxygen uptake, A–V difference and cardiac output was achieved after two to three minutes. A number of exceedingly disabled patients who were unable to raise their cardiac output on exercise and had very abnormally increased A–V difference reached a steady hemodynamic state in a few minutes.

The constancy of the ventilation, respiratory quotient, pulse rate and oxygen uptake were all found to be unreliable criteria of the steadiness of the cardiac output.

The mixed venous blood saturation fell to exceedingly low levels in a number of patients who were, however, able to continue to exercise without undue distress for several minutes. In most cases, there was a remarkably prompt recovery

of the mixed venous blood saturation to resting levels after the exercise stopped.

There appeared to be no correlation between the degree of dyspnoea and the ventilation, either in absolute terms or expressed as a percentage of the maximum breathing capacity. Similarly no important relation was found between the degree of dyspnoea and the level of mixed venous blood saturation, the behavior of the cardiac output, the pulmonary artery pressures, or the resting transpulmonary pressures (P.C.P.)

Brief mention is made of evidence suggesting that there is considerable reduction of blood flow to the upper body, especially to the arms, when these disabled patients exercise.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. G. Cumming and Staff Nurse E. M. Dalton for help in a number of these studies and also to Mr. R. J. Mills for excellent technical assistance. We are indebted to grants from the Endowment Research Fund of the United Birmingham Hospitals and from Messrs. Imperial Chemical Industries Ltd.

APPENDIX

Details of Subjects

Group One

C1. Male. Aged 25. S.A. 1.68 m². Mitral stenosis. Disability Grade I.[4] No symptoms except acute cardiac neurosis seven days before admission when suddenly told he had "serious heart disease."

Normal sized heart. Sinus rhythm. Classical signs of mitral stenosis. *Fluoroscopy:* left atrium normal.

C2. Male. Aged 43. S.A. 1.60 m². Mitral stenosis. Disability Grade 2. Dyspnoea two and one-half years ago, gradually increased. Several severe attacks nocturnal dyspnoea during last year. No hemoptysis or edema.

Heart moderately enlarged. Sinus rhythm. Classical signs of mitral stenosis, slight aortic incompetence. *Fluoroscopy:* left atrium not enlarged. *Operation:* moderate mitral stenosis, no regurgitation.

C3. Female. Aged 42. S.A. 1.60 m². Mitral stenosis and regurgitation. Disability Grade 2. Attacks of paroxysmal tachycardia since 18. Otherwise no symptoms until 10 months ago. After sore throat developed shortness of breath and ankle edema, progressing to frank congestive failure. Rapid recovery, now has excellent exercise tolerance. No hemoptysis.

Heart considerably enlarged. Auricular fibrillation. Signs of mitral stenosis and regurgitation. Slight aortic incompetence. *Fluoroscopy:* left atrium and both ventricles enlarged.

C4. Female. Aged 23. S.A. 1.49 m². Mitral stenosis. Disability Grade 3a. Onset of dyspnoea one year ago, gradually increasing. Occasional hemoptysis last year. No edema.

Heart not enlarged. Sinus rythm (intermittent auricular fibrillation). Classical signs of mitral stenosis with slight aortic incompetence. *Fluoroscopy:* left atrium enlarged. *Operation:* mitral stenosis, no regurgitation.

C5. Male. Aged 34. S.A. 1.61 m². Mitral stenosis. Disability Grade 3a. Laborer's work until two months ago without symptoms, then dyspnoea on exertion and mild ankle edema on several occasions. No frank congestive failure. No hemoptysis.

Heart greatly enlarged. Auricular fibrillation. Moderate mitral systolic and loud mid-diastolic murmur. *X-ray:* left atrium greatly enlarged. *Operation:* moderate mitral stenosis no regurgitation.

Group Two

C6. Female. Aged 36. S.A. 1.52 m². Mitral regurgitation. Disability Grade 2. Some dyspnoea since childhood with slight deterioration recently. Considerable variation. No edema or hemoptysis at any time.

Heart moderately enlarged. Sinus rhythm. Left ventricular impulse increased with right ventricular thrust. Loud systolic murmur conducted backwards and soft mid-diastolic murmur in mitral area. No aortic murmurs. *Fluoroscopy:* large left atrium with systolic expansion. Left ventricle enlarged but right ventricle more so. Calcified mitral valve. Considerable mitral regurgitation considered present. Operation not advised.

C7. Female. Aged 35. S.A. 1.58 m². Mitral stenosis. Disability Grade 3b. Dyspnoea ten years with considerable deterioration last two years. Two slight hemoptyses recently. No edema.

Heart slightly enlarged. Sinus rhythm. Classical signs of mitral stenosis. *X-ray:* left atrium enlarged. *Operation:* considerably stenosed, funnel shaped mitral valve.

C8. Female. Aged 44. S.A. 1.61 m². Mitral stenosis. Disability Grade 3b. Disability heralded by attack of congestive failure five years ago. No edema or hemoptysis since but has remained severely disabled by dyspnoea.

Heart considerably enlarged. Sinus rhythm. Classical signs of mitral stenosis with soft apical systolic murmur and slight aortic incompetence. *X-ray:* big heart, predominantly right ventricle. Left atrium also enlarged. *Operation:* very severe mitral stenosis.

C9. Female. Aged 24. S.A. 1.45 m². Mitral stenosis. Disability Grade 3b. First noticed slight dyspnoea 10

[4] The clinical grading of disability used here is in accordance with that of the American Heart Association, except that Grade 3 is considered by the authors to be too wide, as it includes all degrees of disability between slight discomfort with normal activities, to discomfort at rest. It has, therefore, been subdivided into Grades 3a and 3b. Patients with Grade 3a disability are able to walk a mile or more on the flat at their own speed and can ascend stairs slowly without undue discomfort. Many such patients can still earn a livelihood or perform most household tasks.

years ago. More severe last four years with considerable deterioration in last nine months. No edema or hemoptysis.

Heart very slightly enlarged. Sinus rhythm. Classical signs of mitral stenosis. *X-ray:* Heart slightly enlarged due to right ventricular hypertrophy. *Operation:* severe mitral stenosis, no regurgitation.

C10. Male. Aged 34. S.A. 1.59 m^2. Mitral stenosis. Disability Grade 3b. Some dyspnoea 10 years ago on violent exertion when successful paratrooper. Increased slowly since, but more rapid deterioration last three years and especially last six months. Several attacks of severe nocturnal dyspnoea recently and hemoptysis once. No edema. Anemic (Hb. 8.25 Gm. per 100 ml.)

Heart only slightly enlarged. Sinus rhythm. Classical signs of mitral stenosis. Soft aortic diastolic murmur. *X-ray:* heart within limits of normal but pulmonary conus enlarged. *Operation:* delayed 9 months for possible subacute rheumatism to subside. Anemia responded to iron treatment. Severe mitral stenosis found, no regurgitation.

C11. Female. Aged 28. S.A. 1.46 m^2. Mitral stenosis and regurgitation Disability Grade 3b. Dyspnoea commenced five years ago. Two years ago "pneumonia" and has deteriorated rapidly since. Severe nocturnal dyspnoea and hemoptysis. No edema.

Heart considerably enlarged. Auricular fibrillation. Mitral systolic murmur conducted backwards with rumbling diastolic murmur *X-ray:* left atrium enlarged considerably. *Operation:* mitral valve stenosed and calcified with considerable regurgitation.

C12. Male. Aged 30. S.A. 1.68 m^2. Mitral and aortic stenosis. Disability Grade 4. Well until year ago when developed congestive cardiac failure with severe recurrent pulmonary infarction. Recurrent congestive failure since. No edema at time of study, although venous pressure raised. Hemoptysis related to pulmonary infarction.

Large heart, right and left ventricular enlargement. Auricular fibrillation. Soft mitral systolic and mid-diastolic murmurs. Marked aortic systolic murmur conducted into neck and soft diastolic murmur at aortic area. *Fluoroscopy:* large left atrium, much enlargement of right ventricle, some enlargement of left ventricle. *Died after thoracotomy.* Severe mitral stenosis. Moderate aortic stenosis.

Group Three

C13. Male. Aged 47. S.A. 1.90 m^2. Mitral regurgitation. Disability Grade 3b. First noticed undue shortness of breath 15 months ago. Deteriorated steadily since, being now gravely disabled. No edema or hemoptysis.

Very large heart. Auricular fibrillation. Mitral systolic thrill and murmur, well conducted backwards. Mitral mid-diastolic murmur. *X-ray:* Right ventricle enlarged. Left ventricle slightly enlarged. Right atrium enlarged. Mitral valve calcified. *Operation:* rigid calcified valve with gross regurgitation.

C14. Female. Aged 36. S.A. 1.65 m^2. Mitral stenosis. Disability Grade 3b. Slight shortness of breath on exertion 17 years ago, becoming slowly but progressively worse. Now very handicapped. Ankle edema during last year. Hemoptyses last few months.

Heart only slightly enlarged. Auricular fibrillation. Moderate mitral systolic and loud mid-diastolic mitral murmurs. *Operation:* large left atrium. Moderately severe mitral stenosis with some regurgitation.

C15. Female. Aged 39. S.A. 1.65 m^2. Mitral stenosis. Disability Grade 4. Fit till three years ago when febrile illness with joint pains heralded onset of exertional dyspnoea and ankle edema. Has deteriorated steadily since. Attacks of nocturnal dyspnoea for one year. Hemoptysis once only.

Heart only slightly enlarged. Auricular fibrillation. Loud mitral mid-diastolic murmur. *Fluoroscopy:* left atrium and both ventricles enlarged. *Operation:* severe mitral stenosis, no regurgitation.

C16. Male. Aged 34. S.A. 1.76 m^2. Mitral and aortic stenosis. Disability Grade 4. Dyspnoea on exertion commenced five years ago. Deteriorated rapidly next two years and for last three years has had recurrent congestive cardiac failure. No hemoptysis. No edema at time of study.

Enormous heart. Auricular fibrillation. Clear signs of mitral stenosis, aortic stenosis and slight regurgitation, and intermittent functional tricuspid regurgitation. Systolic murmur at apex, difficult to dissociate from aortic murmur, but some conduction into axilla. *Fluoroscopy:* all chambers of heart enlarged. *Operation:* severe mitral stenosis found; no regurgitation.

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THE EXCRETION OF GLUCURONIC ACID AND OF ACID MUCOPOLYSACCHARIDES IN NORMAL HUMAN URINE [1]

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The urinary excretion of acid mucopolysaccharides has been a subject of controversy. Acid mucopolysaccharides are polymers of disaccharides composed of equimolar portions of N-acetyl or N-sulfate hexosamine and hexuronic acid, occurring either free or as esters of sulfuric acid and in loose combination with protein substances. They include such substances as chondroitin sulfate, heparin and hyaluronate. The evidence for and against the urinary excretion of chondroitin sulfate was reviewed briefly by Oliver (1) whose own experiments led him to the conclusion that an "X-body," similar in some of its characteristics to chondroitin sulfate, was one of several urine constituents which influenced the precipitation of urinary proteins. Astrup (2) was particularly interested in the excretion of heparin and devised a method for extracting from urine metachromatic substances which would inhibit clotting. His method was later used by Jacques, Napke, and Levy (3) to study in greater detail the recovery of injected heparin from the urine of humans and various animals. Metachromatic material obtained from normal urine was noted by these investigators to be so small in amount that its presence did not interfere with their study of additional metachromatic substances excreted after the injection of heparin.

In studying acid mucopolysaccharides, one must to some extent be concerned with their hydrolytic products. One of the important components of most [2] acid mucopolysaccharides is hexuronic acid. For reasons of convenience and interest, this component has been emphasized considerably more in the present studies than has the equally important hexosamine component. The metabolism of glucuronic acid is not well understood. Mosbach and King (5) and Eisenberg and Gurin (6) have contributed useful data based on tracer studies, and Levvy (7) has reviewed the available material on the role of β-glucuronidase and related enzymes. Fishman, Smith, Thompson, Bonner, Kasdon, and Homburger (8) studied the effect of the ingestion of glucuronolactone on the blood levels of glucuronic acid in humans. They recorded the urinary excretion of glucuronic acid, also, in a few patients who were given glucuronolactone by mouth and concluded that little of the substance was excreted in the urine. Ormsby (9) also noted that urinary excretion of glucuronic acid accounted for only 15 to 20 per cent of an ingested dose of glucuronolactone in man. The total excretion of glucuronic acid in the urine represents the very small portion which occurs as a constituent of the acid mucopolysaccharides but also the much larger portion which is excreted as a constituent of a variety of other substances unrelated to acid mucopolysaccharides.

The present study is concerned chiefly with acid mucopolysaccharides isolated from the urine of normal humans by the method of Astrup (2). Quantitative studies were based on the measurement of glucuronic acid following acid hydrolysis of the Astrup fraction. Qualitative studies were chromatographic in nature and demonstrated the presence in the fraction of a substance showing the same R_f value as chondroitin sulfate. Ingestion of glucuronolactone was shown to increase significantly not only the direct excretion of glucuronic acid in urine but also the excretion of acid mucopolysaccharides.

[1] This investigation was supported in part by grants from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service; Baxter Laboratories, Inc.; Duke University Research Council.

[2] Meyer, Linker, Davidson, and Weissmann (4) described a mucopolysaccharide (keratosulfate) of bovine cornea which is composed of N-acetylglucosamine, galactose, and sulfate.

METHODS

Subjects. Subjects were personnel associated with the laboratory. Collections of 24-hour urines were not always on consecutive days during the period of baseline observations. Baseline collections always included, however, at least two days immediately prior to the ingestion of glucuronolactone and two to three days immediately following cessation of the drug. Glucuronolactone was administered in a single 5-gram oral dose on each of three to six consecutive days.

The patient on whom the data of Table I were obtained was an otherwise healthy young white male with a chronic duodenal ulcer.

TABLE I

Effect of prostatic secretions on urinary content of glucuronic acid and acid mucopolysaccharides

Normal ♂, white, aet 31	Mg. of glucuronic acid per 100 ml. of urine	Mg. of glucuronic acid in Astrup fraction per 100 ml. of urine
Before prostatic massage	59.0	0.907
After prostatic massage	57.5	0.849

TABLE II

Glucuronic acid and acid mucopolysaccharide content of urine obtained from renal pelvis of patient, post-nephrostomy, with previous unilateral nephrectomy

Post-operative day	Mg. glucuronic acid per 24-hour urine	Mg. glucuronic acid in Astrup fraction of 24-hour urine
1	1,411	7.20
2	787	11.98
3	255	7.23
4	178	6.59
5	106	4.26

The patient on whom the data of Table II were obtained was a 67-year old colored woman with a previous unilateral nephrectomy, on whom a nephrostomy was done. The 24-hour urine collections from the nephrostomy tube constituted, therefore, the patient's total urinary output. Collections were terminated when evidence of urinary tract infection appeared post-operatively.

Isolation of acid mucopolysaccharides from urine. The method of Astrup (2) was followed closely.[3]

In patients eating diets which impose rigid sodium restriction, it has been found necessary to add additional salts to the urine prior to using benzidine HCl in order to obtain maximal precipitation of acid mucopolysaccharides. For the sake of uniformity, this step has been used subsequently routinely with urine from both patients and a new series of controls. The yield of Astrup fraction from controls on ordinary diets is somewhat greater by this means than by the method used in the present study.

Quantitative studies of the acid mucopolysaccharide fraction. The glucuronic acid content of the Astrup fraction was determined by acid hydrolysis and subsequent production of the carbazole color reaction of Dische (10). Quadruplicate tubes for hydrolysis were set up on each Astrup fraction, one tube later serving as a blank, receiving 0.2 ml. of alcohol instead of 0.2 ml. of an alcoholic solution of carbazole. DuPont Sulfuric Acid C. P. Reagent was used.[4] A standard curve based on readings obtained with glucuronolactone in duplicate 20, 30, and 40 gamma amounts was included in every set of determinations throughout the studies. Samples were discarded if the highest and lowest of the readings (optical densities at 530 λ on a Coleman Junior Spectrophotometer) on the triplicate hydrolysates failed to check within 15 per cent of each other. The entire run was discarded if the duplicate readings on the standard curve failed to check within 15 per cent or if the standard curve did not pass within 0.035 of the zero point.

Qualitative studies of the acid mucopolysaccharide fraction. Chromatographic studies were carried out, using $15 \times 22\text{-}\frac{1}{2}$ inch sheets of Whatman No. 1 filter paper, ascending, six sheets per run in a chromatocab. The solvents used were propanol or ethanol in pH 6.5 M/15 phosphate buffer for 72 and 48 hours, respectively, at 76° F. The concentrations of alcohol used are indicated in the accompanying Figures 5 and 6. The known substances with which the urine samples were compared were chondroitin sulfate (General Biochemicals, Inc.) and Heparin Sodium (Liquaemin; Organon, Inc.) each applied one time to the filter paper, in concentration of 5 and 6 mg. per ml. of distilled water, respectively (11).[5]

The urine samples were prepared for chromatography as follows: The Astrup fraction containing acid mucopolysaccharides from 50 ml. of urine was taken up in 5 to 10 ml. of distilled water and dialyzed first against 20 volumes of distilled water with constant shaking for 15 to 16 hours at 5° C., then against 70 ml. of 20 per cent aqueous polyvinylpyrrolidone with constant rotation in a cylinder for 24 hours at 7° C. The contents of the dialysate casing were then taken up in 0.4 ml. of distilled water, yielding a small amount of very slightly viscid fluid material varying in appearance from clear colorless to clear or turbid yellow or brown. On filter paper the material stained metachromatically with toluidine blue. For chromatographic runs, it was usually applied three times to the starting point, with drying between appli-

[3] By this method, not less than 80 per cent of added heparin (300 mcg. per 50 ml. of urine) could be recovered consistently.

[4] A green color resulted from the use of several other brands of commercial sulfuric acid tried.

[5] Subsequent to the publication of (11), the interesting study of Molho and Molho-Lacroix (12) has come to the author's attention, in which chromatograms were achieved on filter paper by the use of a propanol-water solvent for aqueous heparin and an acetone-water solvent for heparin added to plasma.

cations. It was necessary that the material be present in sufficient concentration to stain metachromatically, but overloading caused streaking. The attempt was to achieve maximal concentration without encountering overloading effects.

The chromatograms were developed by a technique developed in this laboratory and similar to that used by Rienits (13). The technique is reported in detail elsewhere (14). The major technical differences from Rienits' method consist of the fixation of the water-soluble materials on the paper prior to staining with aqueous toluidine blue and then much more complete rinsing in water which has been acidified with glacial acetic acid. The resultant metachromatic spots are quite stable against a blue background.

Quantitative studies of the total urinary output of glucuronic acid. The total excretion of glucuronic acid in urine was determined, also, using appropriate dilutions of urine in the colorimetric method of Dische (10). These were set up always in triplicate tubes for hydrolysis, using one, later, for a color blank, as before.

RESULTS

Results are graphically summarized in Figures 1 to 4. The difference in excretion of acid muco-

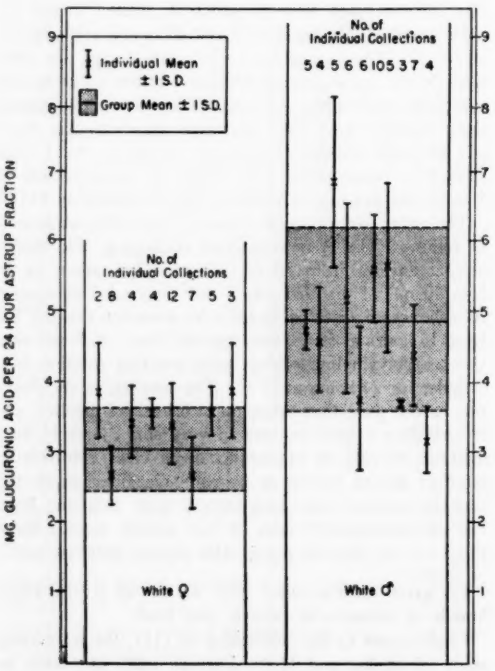
Individual Mean ± 1 S.D.
Group Mean ± 1 S.D.
No. of Individual Collections
2 8 4 4 12 7 5 3
No. of Individual Collections
5 4 5 6 6 10 5 3 7 4
MG. GLUCURONIC ACID PER 24 HOUR ASTRUP FRACTION
White ♀
White ♂

FIG. 1. 24 HOUR URINARY EXCRETION OF ACID MUCOPOLYSACCHARIDES (EXPRESSED AS MG. OF GLUCURONIC ACID) BY NORMAL HUMAN ADULTS

FIG. 1

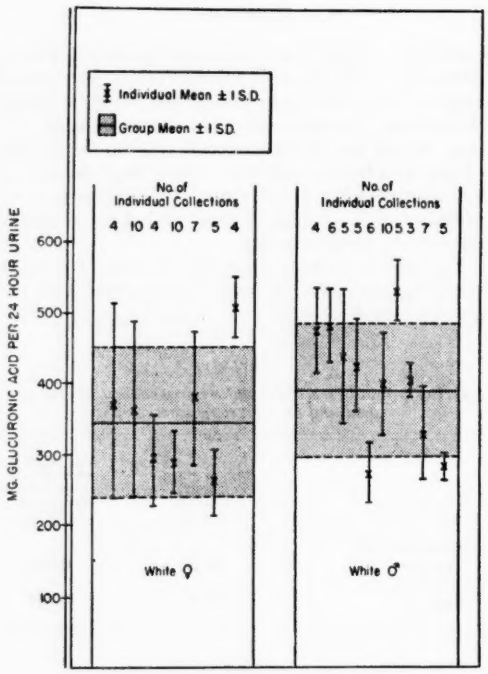
Individual Mean ± 1 S.D.
Group Mean ± 1 S.D.
No. of Individual Collections
4 10 4 10 7 5 4
No. of Individual Collections
4 6 5 5 6 10 5 3 7 5
MG. GLUCURONIC ACID PER 24 HOUR URINE
White ♀
White ♂

FIG. 2. 24 HOUR URINARY EXCRETION OF GLUCURONIC ACID (IN MG) BY NORMAL HUMAN ADULTS

FIG. 2

polysaccharides (expressed as mg. of glucuronic acid after hydrolysis) between normal white males and females (Figure 1) is of high statistical significance ($p = < 0.01$). This is in contrast to the excretion of total glucuronic acid which is essentially the same in white males and females. (Figure 2).

Because of the greater individual standard deviation in excretion of acid mucopolysaccharides by males as compared to females, the possibility of a prostatic derivation of part of the material was entertained. Table I illustrates data on one patient which fail to support this likelihood. The observations were repeated in another patient with similar results.

Data on molecular size of acid mucopolysaccharides are conflicting but some estimates range as high as 200,000 to 400,000 (15). Glomerular filtration in this form, then would be improbable. However, Table II illustrates the presence of these

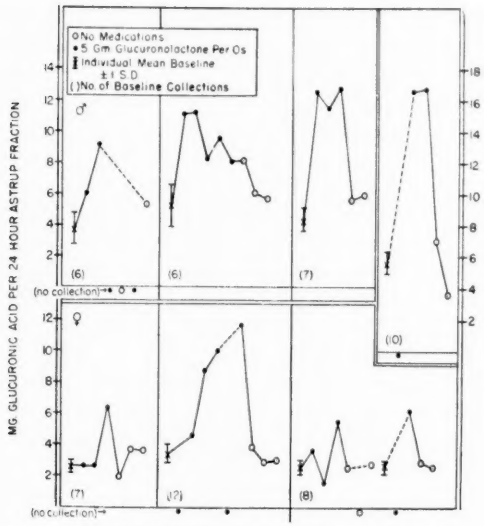

FIG. 3 EFFECT OF INGESTION OF GLUCURONIC ACID ON THE 24 HOUR URINARY EXRETION OF ACID MUCOPOLYSACCHARIDES

FIG. 3

The abscissae represent successive days on which 24-hour collections of urine were made during and following the experimental period of ingestion of glucuronolactone.

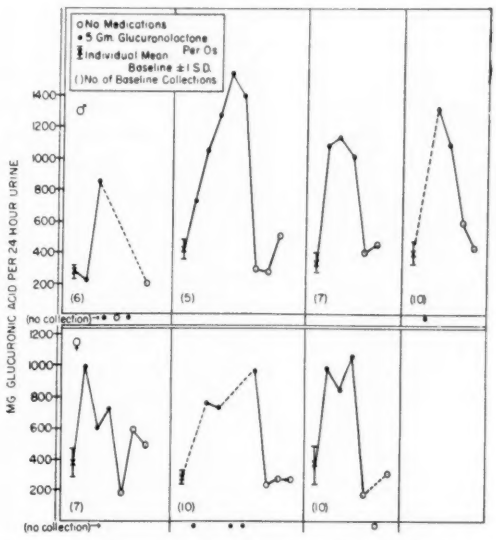

FIG. 4 EFFECT OF INGESTION OF GLUCURONIC ACID ON THE URINARY EXCRETION OF GLUCURONIC ACID

FIG. 4

The abscissae are as for Figure 3.

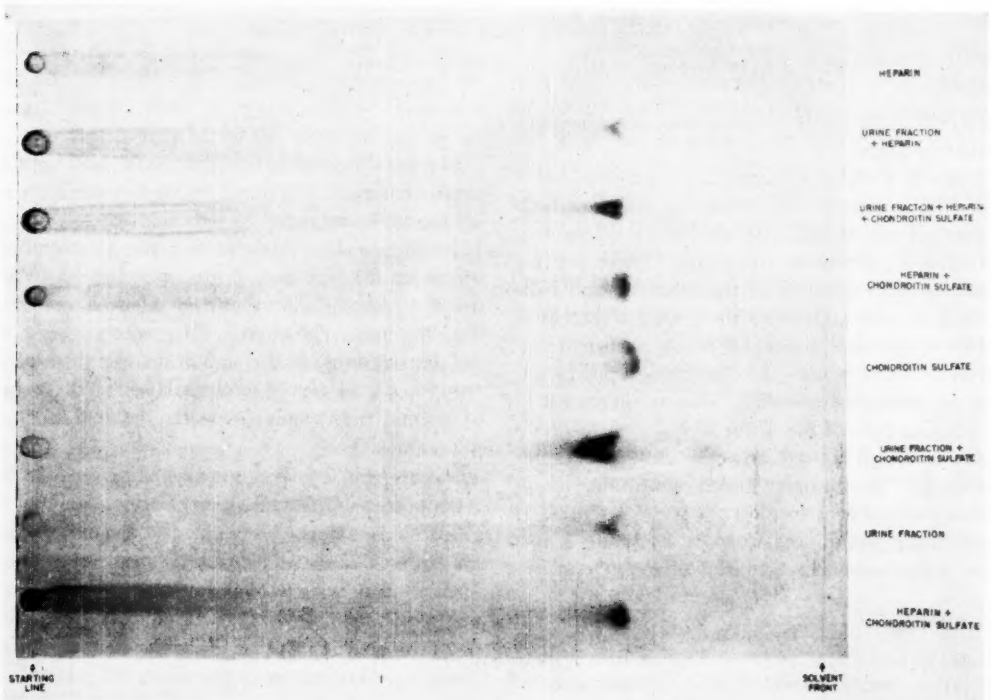

FIG. 5. URINE FRACTION FROM NORMAL WHITE FEMALE

Solvent 52 per cent ethanol in pH 6.5 M/15 phosphate buffer.

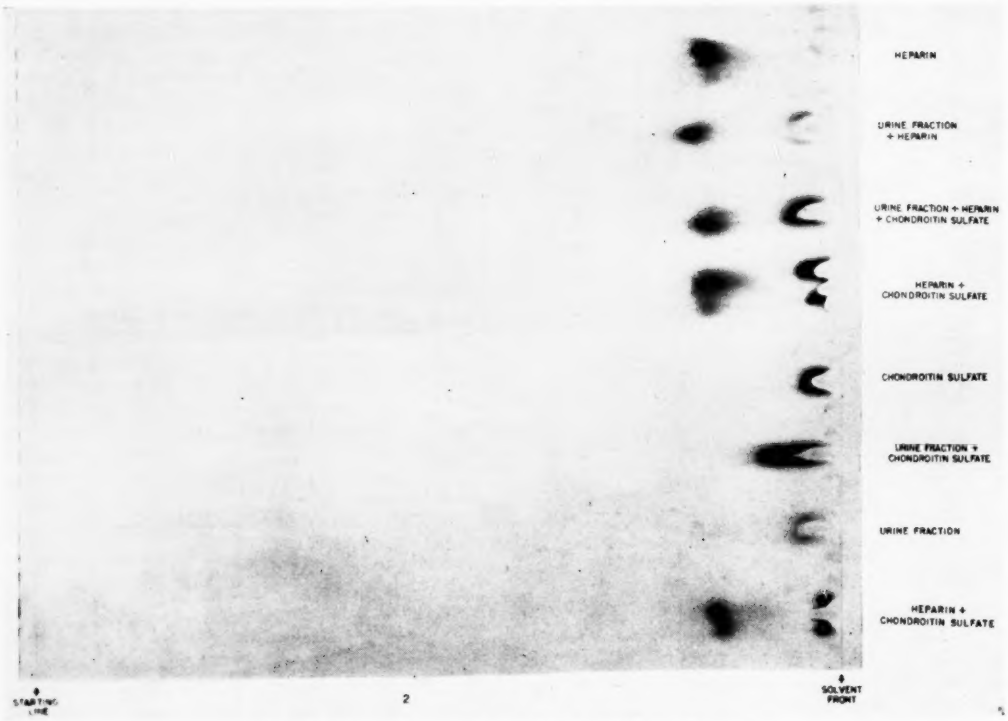

FIG. 6. URINE FRACTION FROM NORMAL WHITE FEMALE

Solvent 37 per cent propanol in pH 6.5 M/15 phosphate buffer.

materials in the renal pelvis in amounts which are relatively high.[6]

Figures 5 and 6 illustrate chromatograms of a urine sample from one normal female, run both separately and mixed with the known substances, chondroitin sulfate and heparin. These are essentially representative of the other normal individuals so tested, although there were instances in which it appeared impossible to add sufficient material for a spot which could be photographed without encountering streaking due to overloading. A urine sample of the nephrostomy patient previously described showed a similar chromatographic pattern. The chromatograms show the major urinary component to have the same R_f value as chondroitin sulfate and to move as a single spot when mixed with chrondroitin sulfate.

[6] The possibility which is suggested by the data that there is a rise post-operatively in the excretion of these substances is currently under investigation, both in surgical patients and in stressed non-surgical individuals.

DISCUSSION

The total excretion of glucuronic acid in the urine of normal individuals is of the same order of magnitude as noted by other investigators (9, 16) (Figure 2). A significant rise in excretion occurs on the ingestion of glucuronolactone (Figure 4), although most of the ingested material does disappear elsewhere. Of greater interest in the present study is the significant rise in the excretion of acid mucopolysaccharides in the urine of normal individuals following the ingestion of glucuronolactone. This is not due to a non-specific precipitation of glucuronic acid with the Astrup fraction, because very large amounts of glucuronolactone, menthol glucuronide and borneol glucuronide can be added to urine prior to isolation of the Astrup fraction without altering in any way the subsequent yield of glucuronic acid obtained from the acid mucopolysaccharide fraction. It is possible, therefore, that the ingested glucuronic

acid enters a metabolic pool concerned with acid mucopolysaccharides, the excess being in part excreted in this form. The rise in excretion of acid mucopolysaccharides did not always occur on the first day of ingestion of glucuronic acid. However, no consistent delay could be demonstrated (Figure 3) which might be considered the time necessary for production of the material. Tracer studies would be helpful in this regard. Urine collections over 6-hour periods continuously for six days, including a 2-day period of ingestion of glucuronolactone, yielded data which failed to show any consistent peak of excretion of acid mucopolysaccharides, either during baseline collections or following ingestion of glucuronolactone.

The daily excretion of acid mucopolysaccharides is relatively constant in each individual but varies to a greater degree between individuals. Why both individual and group variations should be greater in males than in females is unexplained at the moment. The wide variations which occur under certain experimental and clinical situations and in certain disease processes form the subject of separate investigations now in progress and beyond the scope of the present report.

The way in which acid mucopolysaccharides get into urine is not certain. The present study demonstrates that the material was present in urine collected from the renal pelvis. This observation plus that of failure to show a rise in urinary acid mucopolysaccharides following prostatic massage make it unlikely that any appreciable amount of the material is added in the lower urinary tract. The data yield no evidence bearing on the question of secretion by the renal tubular epithelial cells. However, the studies of Piper (17) on the fate of heparin injected into rabbits led him to the conclusion that that portion of the heparin which was excreted by way of the kidneys was handled by a combination of glomerular filtration and tubular secretion. Consideration of glomerular filtration requires consideration of molecular size. By streaming birefringence, Blix and Snellman (15) estimated the molecular weight of chondroitin sulfuric acid to be of the order of 260,000. By osmometry, Mathews and Dorfman (18) estimated molecular weights of 15,000 to 43,000 for chondroitin sulfuric acid, variations occurring with variations in method of separating the material for study. Discrepancies, therefore, may result not only from the fact that these substances are probably not pure and of single molecular weight but also because the shape of the molecule may vary in different environments, with consequent effect on estimates of molecular weight by various methods. If materials of large molecular weight are considered in regard to glomerular filtration, the possibility is raised that the materials circulate in the blood in the form of a smaller structural unit which may be filtered through the glomerulus, later polymerizing in the tubules to form the substance isolated in the present study from urine obtained from the renal pelvis. The concept of smaller-sized components of ground substance is not a new one (19). However, since it is still purely speculative and since even the question of original molecular size is unsettled, the advice of Meyer (20) is followed in the avoidance of any debate as to whether such smaller units, if they occur, result from depolymerization, lack of polymerization, or formation of an entirely different unit of similar structure but smaller size than the acid mucopolysaccharides. Of greater interest, perhaps, is consideration of the smaller molecular size estimated by Mathews and Dorfman (18). Acid mucopolysaccharides of molecular weight 43000 or less might quite reasonably be expected to be filtered through the glomerulus. This would raise interesting questions as to whether the bulk of the material is then reabsorbed by the renal tubules as in the case of filtered serum albumin or whether it is excreted as filtered. Present methods fail to detect with certainty acid mucopolysaccharides as such in normal blood. However, the 24-hour excretion of these substances is small and could be accounted for by very minute amounts in plasma filtered at the rate of 180 liters per day.

The character of at least one and apparently the major component present in the acid mucopolysaccharide fraction from human urine is on more solid ground. Although the identity of the component is not fully established, it does exhibit the same R_f value chromatographically in a single solvent system as does chondroitin sulphate, and it moves as a single spot chromatographically when mixed with chondroitin sulphate.

SUMMARY

The mean 24-hour excretion of glucuronic acid in normal human urine was 345 mg. ± 44 (S.D.) in white females and 391 mg. ± 56 in white males. There is no significant difference between the two values.

The mean 24-hour excretion of acid mucopolysaccharides (expressed as glucuronic acid after hydrolysis) in normal human urine was 3.0 mg. ± 0.6 (S.D.) in white females and 4.9 mg. ± 1.3 in white males. The difference between the two values was significant ($p = < 0.01$). Prostatic massage did not increase the amount of the material in urine. The material was present in urine obtained from the renal pelvis of one patient and thus was not derived to any significant degree in that patient from structures lower in the urinary tract.

The urinary excretion of acid mucopolysaccharides by humans was markedly increased by the ingestion of 5 Gm. of glucuronolactone daily for several days.

Chromatographic studies of a further purified fraction of acid mucopolysaccharides from normal human urine showed the major component of the fraction to have the same R_f value as chondroitin sulfate and to produce a single spot when mixed with chondroitin sulfate, in a single solvent system.

ACKNOWLEDGMENT

This work was done with the technical assistance of Mrs. Dorothy Bilancio.

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AN ACCELERATING PROPERTY OF PLASMA FOR THE COAGULATION OF FIBRINOGEN BY THROMBIN [1, 2]

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The last step in the clotting process is the conversion of a soluble protein, fibrinogen, into an insoluble protein, fibrin. This conversion takes place as the result of the action of an enzyme, thrombin. Among the many factors determining the rate of this reaction are the concentrations of fibrinogen and thrombin, the ionic strength of the medium, and the presence or absence of certain bivalent cations.

Biggs and Macfarlane (1) observed that the clotting time of mixtures of oxalated plasma and thrombin varied in different conditions. They suggested the possibility that there are factors in plasma which accelerate the thrombin-fibrinogen reaction. In the present report, such a factor, present in normal plasma, and capable of accelerating the conversion of fibrinogen to fibrin is described. Some of the characteristics of this accelerating property of plasma will be discussed.

MATERIALS

Oxalated human plasma was obtained by mixing venous blood with one-ninth its volume of 0.1M sodium oxalate solution and centrifuging the mixture at room temperature for 15 minutes at 2,500 rpm in a Size 1, Type SB International centrifuge. The plasma was stored between 0° and 4° C. until used. In some experiments, the plasma was shaken for 10 minutes with a carboxylic acid ion exchange resin, Amberlite IRC-50 (Rohm and Haas) in the sodium phase (pH 7.7), in order to remove bivalent cations.

Commercial bovine *thrombin* (Parke, Davis and Co.) was dissolved in barbital-saline *buffer* in a concentration of 1,000 National Institutes of Health (N.I.H.) units per ml. (2) and stored at −25° C. until used. Amberlite-treated thrombin was prepared in the same manner as amberlite-treated plasma. The clotting time, using this calcium-deficient thrombin, was somewhat longer than with untreated thrombin.

The *buffer* used was 0.025M barbital in 0.125M sodium chloride solution, at pH 7.5, prepared by dissolving 2.76 Gm. of barbital, 2.06 Gm. of sodium barbital and 7.30 Gm. of sodium chloride in one liter of water.

Defibrinated plasma was prepared by mixing 100 parts of oxalated plasma with 3 parts of a solution of bovine thrombin containing 1,000 N.I.H. units per ml. The clot which formed was removed either by wrapping it around a glass rod and expressing the defibrinated plasma, or by shaking the contents of the tube with about one-tenth volume of crushed pyrex glass. The fibrin adhered to the glass so that the defibrinated plasma could be decanted. The defibrinated plasma was then incubated at 37° C. for 30 minutes to inactivate the thrombin. The adequacy of defibrination was tested by the addition of fresh thrombin to an aliquot. To test whether the thrombin had been inactivated, the defibrinated plasma was mixed with a solution of bovine fibrinogen; an absence of clotting was taken to mean that the thrombin was no longer active.

Barium sulfate-adsorbed plasma was prepared by mixing oxalated plasma or defibrinated plasma with approximately one-tenth its volume of powdered barium sulfate (Baker). The mixture was incubated at 37° C. for 10 minutes with frequent stirring, and the plasma was then separated by centrifugation for 15 minutes at 2,500 rpm in the International centrifuge.

Dialyzed plasma or its fractions was prepared by dialysis for 16 to 24 hours in cellophane sacs (Visking No-Jax sausage casings, one inch in diameter) at 4° C. against 0.15M sodium chloride solution. When the solutions contained ammonium sulfate, they were first dialyzed against running cold tap water for several hours before dialysis against sodium chloride. The term *dialysate* will be used to define the solution of sodium chloride against which the plasma or its fraction had been dialyzed.

Crude *fractions of plasma* were prepared by the addition of solid ammonium sulfate to plasma which had first been defibrinated and adsorbed with barium sulfate. Each mixture of ammonium sulfate and plasma was kept at 0° C. for 30 minutes. The precipitate which formed was separated by centrifugation at 4,500 rpm. for 5 minutes at −5° C. in a Servall Type SS-1 angle centrifuge. In this manner, the plasma was separated into a fraction precipitated at 33 per cent saturation with ammonium sulfate, a fraction precipitated between 33 and 60 per cent saturation, and a fraction not precipitated with 60 per cent saturation. The two precipitates were each dissolved in a volume of water equal to that of the original

[1] This study was supported by a research grant (P.H.S. No. H1661C) from the National Heart Institute of the National Institutes of Health, Public Health Service.

[2] Presented at the 26th Annual Session of the Central Society for Clinical Research, October 31, 1953.

volume of plasma, and the ammonium sulfate was then removed by dialysis.

The fraction of plasma precipitated between 33 and 60 per cent ammonium sulfate was refractionated by acidification to pH 4.8 with sodium acetate buffer of pH 4.0 and ionic strength 0.15. The acidified fraction was diluted to ionic strength 0.10, cooled to − 5° C., and ethyl alcohol added drop-wise, with stirring, to a concentration of 18 per cent. The mixture was allowed to stand for 30 minutes at − 5° C. and then separated by centrifugation at − 5° C. in the angle centrifuge in the manner described. The concentration of alcohol was then increased to 45 per cent, and the precipitate collected in the same manner. The precipitates were dissolved in a volume of 0.15M sodium chloride solution equal to that of the original plasma. When the activity of such fractions was to be compared with the activity of the ammonium sulfate fractions, the alcohol precipitates were dissolved in the sodium chloride solution against which the ammonium sulfate-precipitated fractions had been dialyzed.

Fractions of human plasma prepared by the low-temperature-ethanol method of Cohn [3] were dissolved in buffer in a concentration of 5 or 10 per cent and serially diluted with buffer. The insoluble material in fractions II–III and IV–1 was removed by centrifugation.

Bovine fibrinogen [4] prepared by the method of Ware, Guest, and Seegers (3) was dissolved in buffer and undissolved residue was separated by filtration through Whatman No. 1 filter paper. In all experiments, a portion of dried fibrinogen weighing 100 mg. was dissolved in 20 ml. of buffer. This preparation contained 0.9 Gm. of sodium chloride for every 3.1 Gm. of protein. Assay of the filtrate demonstrated approximately 300 mg. of coagulable protein per 100 ml. of solution. There was a variation of as much as 50 mg. of coagulable protein per 100 ml. from preparation to preparation, but this did not influence the results obtained. In any given experiment, the same solution of fibrinogen was used throughout.

Heated plasma was prepared by incubating defibrinated plasma in a pyrex glass tube in a water bath. The temperature was measured with a thermometer immersed in the plasma.

METHODS

The clotting time of mixtures of oxalated plasma and bovine thrombin, to be designated as the *thrombin time*, was measured in a 37° C. water bath by adding 0.2 ml. of thrombin to 0.2 ml. of plasma in a pyrex test tube with an internal diameter of 8 mm. The tubes were tilted until clotting occurred. When the thrombin time was 30 seconds or less, duplicate determinations usually checked within two seconds. When the thrombin time was 50 seconds or less, duplicate determinations usually checked within 3 or 4 seconds. When the thrombin time was greater than 60 seconds, the results of duplicate determinations were more erratic.

The following procedure was used to compare normal and pathologic plasma. The activity of solutions of thrombin varied from preparation to preparation. To obviate this difficulty, the thrombin time of each plasma was determined at several concentrations of thrombin. A graph was then drawn of the thrombin time of the normal plasma against the concentration of thrombin, using a logarithmic scale for both the ordinate and abscissa (4). From this graph, the concentration of thrombin which clotted the normal plasma in 30 seconds was determined by interpolation. In the same manner, the thrombin time of the abnormal plasma was plotted as a function of the concentration of thrombin. From this graph, the thrombin time of the abnormal plasma was determined at that concentration of thrombin which clotted normal plasma in 30 seconds. In this manner, the plasma to be tested was compared with a normal plasma, the thrombin time of which was arbitrarily set at 30 seconds. This interpolation of data was used only to render clinical comparison of normal and abnormal plasmas possible. In the majority of the experiments to be described, such interpolation was not necessary to demonstrate the points in question; the actual, rather than the interpolated data are reported.

To determine the effect of different preparations, suitable amounts were added to a sample of plasma or fibrinogen solution and the thrombin time was then determined in the manner described.

RESULTS

The thrombin time of fresh and aged normal oxalated plasma.

In normal subjects, the thrombin time of fresh oxalated plasma was remarkably uniform. In Table I are the relative thrombin times of six normal adults, tested simultaneously. In many other

TABLE I

The thrombin time in normal subjects

Clotting time (sec.) of a mixture of 0.2 ml. of fresh oxalated plasma and 0.2 ml. of bovine thrombin. The relative thrombin times of the plasmas were then determined by interpolation, assuming first that the value for subject No. 1 and then that of the value for subject No. 6 was 30 seconds.

Subject	Thrombin time	
	No. 1 = 30 seconds	No. 6 = 30 seconds
1. G	30	28
2. Si	30	28
3. W	31	29
4. Sm	32.5	30
5. W	32.5	30
6. T	32.5	30

[3] Human plasma fractions were obtained through the courtesy of the late Dr. Edwin Cohn.

[4] Bovine fibrinogen was obtained through the courtesy of Dr. Walter Seegers.

experiments, two or three normal subjects were used as controls. In no instance has the thrombin time among normal individuals differed by more than 4 seconds, when the data were so interpolated that the thrombin time of any given normal plasma was 30 seconds.

In contrast, the thrombin time of normal plasma which had been stored at 4° C. for four months was prolonged compared with fresh plasma. For example, in one experiment the thrombin time of fresh normal plasma was 19 seconds, and of the same amount of aged normal plasma was 43 seconds. The thrombin time of an equal mixture of the two plasmas was 28 seconds, longer than that of the fresh plasma, and shorter than that of the stored plasma.

Moreover, the thrombin time of aged plasma was shortened by the addition of fresh *defibrinated* plasma. Thus the thrombin time of an equal mixture of aged plasma and 0.15M sodium chloride was 77 seconds, while that of an equal mixture of aged plasma and fresh defibrinated plasma was 30 seconds. This makes it seem likely that the prolonged thrombin time of the aged plasma could not be accounted for entirely by a qualitative or quantitative change in its fibrinogen. The fresh plasma appeared to supply some factor other than fibrinogen which shortened the thrombin time of the aged plasma. In contrast, the addition of an equal volume of fresh defibrinated plasma did not significantly alter the thrombin time of fresh oxalated plasma. Subsequent experiments appear to demonstrate that the clot accelerating property of defibrinated plasma is not removed by dialysis.

The thrombin time in pathologic states.

The thrombin time of plasma drawn from patients with a variety of diseases was determined. In hepatic disease, multiple myeloma and eclampsia or severe pre-eclampsia the thrombin time was commonly prolonged (Figure 1). In many of these patients, the hematocrit was below normal, and as a result the proportion of oxalate solution to plasma varied. However, the abnormally prolonged thrombin times in the pathologic plasmas could be demonstrated regularly in plasma which had been dialyzed for 24 hours against 0.15M sodium chloride solution, as well as in plasma which had been treated with a cation exchange resin to substitute sodium ions for other cations.

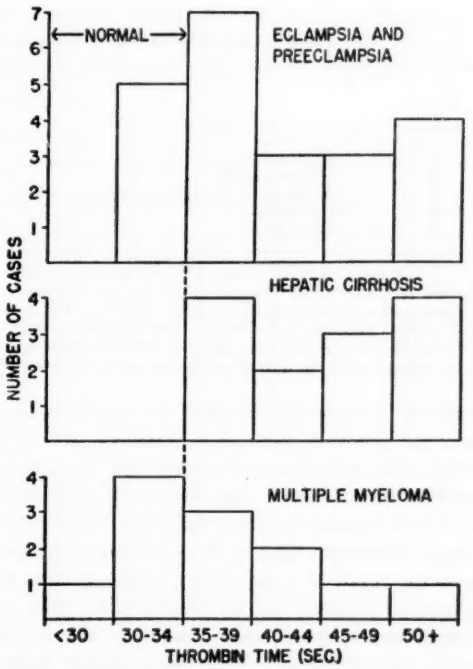

FIG. 1. THE THROMBIN TIME IN MULTIPLE MYELOMA, CIRRHOSIS OF THE LIVER, AND ECLAMPSIA AND SEVERE PRE-ECLAMPSIA

Each plasma was compared with a normal plasma studied simultaneously; the values were interpolated so that the thrombin time of the normal plasma was 30 seconds.

A study of plasmas in which the thrombin time was prolonged supports the view that normal plasma contains an accelerator of the conversion of fibrinogen to fibrin. Normal plasma shortened the thrombin time of abnormal plasma. However, the thrombin time of the mixture was always longer than that of the normal plasma alone. Indeed the thrombin time of an equal mixture of normal and abnormal plasmas was predictable from the thrombin time of each individual plasma. This was determined in the following manner. By interpolation, the amount of thrombin which produced a clot at 30 seconds was measured experimentally for each plasma alone and for the mixture of normal and abnormal plasmas (Table II). At the same time the amount of thrombin which would produce a clot in the equal mixture at 30 seconds was estimated by calculating the average of the

TABLE II

*The effect of mixing normal and abnormal plasmas on the coagulating effect of thrombin**

Patient	Diagnosis	Amount of thrombin which clots plasma in 30 secs.			
		Control	Patient	Equal mixture (experimental)	Equal mixture (calculated)
1	Eclampsia	1.62	2.75	2.23	2.19
2	Pre-eclampsia	1.80	2.70	2.20	2.25
3	Metastatic carcinoma in liver	1.45	2.15	1.92	1.80
4	Cirrhosis	2.08	3.42	3.02	2.75
5	Cirrhosis	2.12	2.90	2.60	2.51

* The amount of thrombin (N.I.H. units) required to clot a mixture of 0.2 ml. fresh oxalated plasma and 0.2 ml. bovine thrombin in 30 seconds at 37° C. The amount of thrombin was determined experimentally for each abnormal plasma and a simultaneously studied control. The amount of thrombin required to clot an equal mixture of abnormal and control plasmas in 30 seconds was determined experimentally. This was compared with the calculated average of the amount of thrombin required to clot each plasma separately. Different solutions of thrombin were used to study each patient, and the data for any one patient cannot be compared with that for other patients.

amount of thrombin required to clot the normal and the abnormal plasmas separately. It will be noted that the calculated result and the experimental result closely approximated each other. For this reason, it seems unlikely that the prolonged thrombin time in these abnormal plasmas was the result of a circulating anticoagulant; the abnormal plasmas had no greater effect than one would expect from mere dilution of the normal with the abnormal. Moreover, unlike normal plasma, fresh plasma from patients with myeloma or eclampsia did not shorten the thrombin time of aged normal plasma. These experiments, then, suggest that the plasma of such patients may be deficient in an accelerating property. They do not rule out the possibility that the accelerator is present but for some reason is physiologically inactive.

Some characteristics of the accelerator in human plasma.

Studies were performed to determine the properties of the substance or substances in normal plasma which shortened the thrombin time of aged or pathologic plasmas. The thrombin time of normal plasma was not altered by adsorption of the plasma with barium sulfate. Prothrombin, serum prothrombin conversion accelerator and Christmas factor are all adsorbed by the barium sulfate. It was therefore possible to study the effect of thrombin on a plasma in which these thrombin-producing factors had been removed. The thrombin time was also not prolonged by dialysis against sodium chloride solution. Indeed, dialyzed plasma had a slightly shorter thrombin time than undialyzed plasma.

The effect of heat on the thrombin time of normal plasma was tested. Fresh oxalated normal plasma was first defibrinated with thrombin to obviate precipitation of fibrinogen by the heating process. This defibrination did not remove the property of plasma which shortened the clotting time of mixtures of plasma and thrombin. The defibrinated plasma was adsorbed with barium sulfate and dialyzed against 0.15M sodium chloride solution for 16 hours at 4° C. A portion of the dialyzed plasma was then heated to 60° C. for 20 minutes, and the effect of unheated and heated dialyzed plasma and of the dialysate upon the thrombin time of normal plasma was measured. In one such experiment, the thrombin time of normal plasma, diluted with an equal volume of the dialysate, was 57 seconds; that of an equal mixture of normal plasma and defibrinated, dialyzed plasma was 42 seconds; and of an equal mixture of normal plasma and defibrinated plasma heated to 60° C. for 20 minutes, was 54 seconds. Thus the accelerating effect of normal defibrinated plasma was diminished by heating it to 60° C. for 20 minutes. Similarly, normal defibrinated plasma heated to 60° C. for 20 minutes lost its ability to shorten the thrombin time of pathologic plasmas.

When plasma was fractionated with ammonium sulfate, the bulk of the accelerating property was present in the fraction precipitated between 33 and 60 per cent saturation (Table III). When this fraction was further subdivided by precipitation with ammonium sulfate, the activity was distributed in each sub-fraction. The fraction precipitated between 33 and 60 per cent saturation was refractionated by precipitation with ethanol at low temperatures. The major portion of the ac-

TABLE III

The effect of plasma fractions on the thrombin time *

Fraction	Thrombin time (sec.)
Saline dialysate	45
Normal dialyzed plasma	26
0–33% ammonium sulfate precipitate	41
33–60% ammonium sulfate precipitate	26
Supernate of 33–60% precipitation	37
0–18% ethanol precipitate	36
18–45% ethanol precipitate	28

* Clotting time (sec.) of a mixture of 0.1 ml. fresh oxalated plasma from a patient with eclampsia, 0.1 ml. of the fraction to be tested, and 0.2 ml. of bovine thrombin (5 units/ml). The fractions were prepared from oxalated normal plasma which had been defibrinated and adsorbed with barium sulfate. The ethanol fractions were re-precipitated from the fraction precipitated between 33 and 60 per cent ammonium sulfate.

tivity was now present in the portion precipitated between 18 and 45 per cent ethanol (Table III). These fractions were effective when mixed with portions of normal or abnormal plasmas. When either defibrinated plasma or the fractions which were prepared were added to bovine fibrinogen solutions, no acceleration of the thrombin time was noted.

The effect of proteins fractionated by the low temperature-ethanol method of Cohn was tested on a solution of human fraction I, on bovine fibrinogen solutions, and on normal and abnormal human oxalated plasma. Fractions III, IV-1, IV-4, IV-7, and V accelerated the thrombin time of oxalated human plasma from a patient with lupus erythematosis (Table IV). They were without appreciable effect on normal oxalated plasma, and were actually slightly inhibitory on bovine fibrinogen solutions. None of the fractions tested had any effect upon the thrombin time of solutions of human Fraction I.

TABLE IV

The effect of Cohn's fractions of normal plasma on the thrombin time of abnormal plasma

Clotting time (sec.) of a mixture of 0.1 ml. fresh oxalated plasma from a patient with lupus erythematosus, 0.1 ml. fraction tested, and 0.2 ml. bovine thrombin (5 N.I.H. units/ml.).

Fraction	Final concentration of fraction (*Gm./100 ml.*)						
	2.5	*1.25*	*0.625*	*0.312*	*0.156*	*0.08*	*0*
II	22	22	22	22	22	21	24
II–III	—	19	19	22	21	21	
III	14	16	18	20	21	22	
IV–1	—	18	19	21	21	22	
IV–4	14	16	21	19	23	23	
IV–7	—	15	16	20	21	24	
V	14	15	17	19	24	25	

The effect of calcium upon the thrombin time.

The reaction between fibrinogen and thrombin is accelerated by the presence of calcium ions (1, 2, 5, 6). Experiments were performed to determine the influence of calcium ions on the thrombin time of abnormal plasma. Oxalated plasma and thrombin solutions were treated with amberlite IRC-50 in the sodium phase to remove bivalent cations. Calcium chloride in varying concentration was then mixed with the thrombin, and the mixture in turn added to the plasma. In some experiments, the plasma was first adsorbed with barium sulfate to remove prothrombin, serum prothrombin conversion accelerator and Christmas factor. In this way, the effect of calcium on the thrombin time was studied independently of any effect that this ion might have on the conversion of prothrombin to thrombin.

TABLE V

The effect of calcium chloride on the thrombin time

Clotting time (sec.) of a mixture of 0.2 ml. of oxalated plasma and 0.2 ml. of an equal mixture of bovine thrombin (20 N.I.H. units/ml.) and calcium chloride solution. The plasma had been adsorbed with barium sulfate. The plasma and thrombin had been treated with amberlite IRC50 to remove bivalent cations. The calcium chloride solutions were diluted serially with 0.15M sodium chloride to maintain a constant ionic strength.

Plasma	Final concentration of calcium chloride (*M/liter*)						
	0.0125	*0.0062*	*0.0031*	*0.0015*	*0.0007*	*0.0004*	*0*
Normal	14	26	27	30	39	48	180
Cirrhosis	18	34	44	52	62	86	∞

These studies demonstrated that calcium shortened the thrombin time of abnormal as well as normal plasmas. In some instances, this effect was sufficiently pronounced that no significant difference between the normal and abnormal plasmas was detected. In others, the thrombin time was still prolonged relative to that of normal plasma even though the concentration of calcium ions was as high as 0.06M or more, an amount far in excess of that present in the circulating blood (Table V).

DISCUSSION

Normal human plasma appears to have the property of accelerating the coagulation of fibrinogen by thrombin. This accelerating property was not removed by defibrination by thrombin, by dialysis against saline solution, nor by adsorption of the plasma with barium sulfate. The accelerating property was concentrated in that fraction of plasma which is precipitated between 33 and 60 per cent saturation with ammonium sulfate. In turn, the accelerator could be reprecipitated at a concentration of alcohol above 18 per cent at − 5° C. and ionic strength 0.1. The accelerating property was diminished in plasma which had been stored at 4° C. for several months, and in fresh plasma which had been heated to 60° C. for 20 minutes. These characteristics seem to distinguish the accelerator of the conversion of fibrinogen to fibrin from prothrombin, serum prothrombin conversion accelerator, Christmas factor, plasma thromboplastinogen, accelerator globulin, and fibrinogen itself.

Seegers and Smith (2) have shown that solutions of acacia will shorten the clotting time of mixtures of fibrinogen and thrombin. Whether the plasma accelerating property which has been described is due to the presence of a specific non-dialyzable substance, or is a non-specific colloidal property of the plasma proteins is not known. It is significant that, unlike acacia, neither the fractions prepared here nor those of Cohn accelerated the coagulation of purified fibrinogen by thrombin. This suggests that the mechanism by which plasma accelerates the clotting of fibrinogen is probably indirect. Perhaps it exerts its action by neutralizing the naturally existing antithrombins which are found in plasma but not in solutions of fibrinogen. Experiments to determine its mode of action are in progress.

Studies with proteins prepared by the low temperature-ethanol method of Cohn indicated that fractions III, IV, and V shortened the abnormally long thrombin time of the oxalated plasma of a patient with lupus erythematosus, but were without effect on normal plasma or on bovine or human fibrinogen solutions. This observation is susceptible to several interpretations. For example, it is possible that the fractions contained a contaminant which shortened the thrombin time. Another possibility is that the accelerator is coprecipitated with proteins over a broad range of conditions, in the same manner that plasminogen is found in many plasma fractions. One cannot, of course, conclude that the accelerator found in these fractions is necessarily identical with those prepared by ammonium sulfate precipitation.

It is known that bivalent cations will accelerate the conversion of fibrinogen to fibrin (1, 2, 5, 6). However, the data presented seem to preclude the possibility that the accelerating activity described was due to the presence of such cations; defibrinated plasma which had been treated with a cation exchange resin still accelerated the clotting of normal plasma by thrombin. No evidence was obtained concerning the source of the plasma accelerator. Ware, Fahey, and Seegers (7) have described an accelerator of the fibrinogen-thrombin reaction obtained from platelets. However, no differences were observed in the reaction between fibrinogen and thrombin in the plasmas of thrombocytopenic as compared to normal subjects. Moreover, the plasma from which active fractions were obtained had been rendered platelet-deficient by two successive centrifugations, suggesting that the accelerator did not arise from a destruction of platelets *in vitro*.

When bovine thrombin was mixed with oxalated human plasma, the clotting time or thrombin time was strikingly uniform in normal subjects. However, it has been demonstrated (1, 8, 9) that in patients with hepatic disease the thrombin time may be considerably prolonged. In the present study, these observations have been confirmed. Other conditions in which a prolonged thrombin time has been noted include the neonatal state (10), multiple myeloma (11), eclampsia and severe preeclampsia (12), amniotic fluid embolism (13), and lupus erythematosus (14). In contrast, the thrombin time was normal in the plasma of normal parturient women (15), patients with uncomplicated thrombocytopenic purpura (14), hemophilia (14) and some patients with obstructive jaundice (8). Unfortunately the thrombin time has not been normal in a sufficient proportion of those patients with obstructive jaundice who have been tested thus far to justify its use as a differential diagnostic test.

Three possible mechanisms for a prolonged thrombin time were considered. Firstly, the reac-

tion between fibrinogen and thrombin might be slowed by an alteration either in the reactivity of fibrinogen or in the polymerization of the fibrin molecule. Secondly, the reaction between fibrinogen and thrombin might be blocked by the presence of an inhibitor. Thirdly, there might be a deficiency of the accelerating property which has been described.

It seems likely that under different conditions, each of these three mechanisms may be responsible for a prolonged thrombin time. For example, Biggs (10) has reported that fibrinogen prepared from the plasma of the newly-born clotted after a longer time than the fibrinogen of adults, when each was mixed with thrombin. Similarly, in a case of cirrhosis, suggestive evidence was obtained that a prolonged thrombin time was due in part to the presence of fibrinogen which reacted more slowly than normal fibrinogen. Definitive studies could not be made because the patient died. Secondly, Conley, Hartmann, and Morse (16) have described patients in whom the thrombin time was prolonged apparently because of the presence of a circulating anticoagulant. No such cases were observed in the present study. In still other patients with a prolonged thrombin time, the plasma behaved as if it were deficient in the accelerator of the coagulation of fibrinogen by thrombin. However, the experiments which have been described do not preclude the possibility that the accelerator is present but in some way rendered ineffective.

When the plasma and thrombin were mixed with calcium ions, the difference between normal and abnormal plasmas was decreased and in some cases disappeared, as had been reported earlier by Biggs and Macfarlane (1). Calcium ions have been shown to increase the tensile strength of fibrin clots (17, 18) and to decrease the solubility of such clots (19). This change in the fibrin molecule apparently requires the presence of a factor in the serum (20, 21). Recently, Shulman (22) has shown that this factor is present in fraction V of bovine plasma. No information is available as yet whether the factor which decreases the solubility of fibrin and the factor which shortens the thrombin time are the same.

The prolongation of the thrombin time observed in pathologic plasma is, of course, a phenomenon demonstrated *in vitro*. Ordinarily, the thrombin time is measured with oxalated plasma. However, when calcium ions were added in the concentration found in plasma, the thrombin time was still prolonged in many of the patients who were studied. For this reason, it is likely that a defect in the coagulation of fibrinogen by thrombin may be responsible for some instances of the hemorrhagic phenomena which are often observed in patients with liver disease, multiple myeloma, and eclampsia or severe pre-eclampsia.

In a preliminary note, the name "thrombin accelerator" was proposed for the accelerating principle in plasma (23). This name seems inappropriate. No evidence has been obtained that the accelerator affects the interaction between fibrinogen and thrombin; it is entirely possible that its influence is upon the polymerization of fibrin. By analogy with the prothrombin conversion accelerators, it might be called "fibrinogen conversion accelerator," but until more data are available, it might perhaps better go unnamed.

SUMMARY

The thrombin time, that is, the clotting time of a mixture of oxalated human and bovine thrombin, was prolonged in patients with liver disease, multiple myeloma, eclampsia and severe pre-eclampsia. The conditions responsible for the prolongation of the thrombin time were investigated. These studies indicate that normal plasma contains a heat-labile, non-dialyzable component which accelerates the coagulation of fibrinogen by thrombin.

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PRELIMINARY ASSAYS OF PENICILLIN BLOOD LEVELS

Studies undertaken in cooperation with the U. S. Public Health Service are reported on by Smith[2] and O'Brien.[3] Preliminary assays had shown the following penicillin blood levels after single injections of from 2,300,000 to 2,500,000 units of Bicillin: 0.134 unit/cc. for the first week, 0.058 unit/cc. for the second week, and 0.091 unit/cc. for the 16-day period of observation. Daily mean and median blood levels of 0.034 unit/cc. or better were obtained. Owing to the limited period of observation, no further assays were made by these investigators, but they point out that there was no evidence of a rapid decline in blood levels at the end of the assay program.

RESULTS IN EARLY SYPHILIS

In the Smith investigation,[2] 127 patients with darkfield positive primary or secondary syphilis were given a single injection of 2,500,000 units of Bicillin. The group comprised 20 seronegative primary patients, 25 seropositive primary, and 82 secondary syphilitic. All patients with seronegative primary syphilis remained seronegative, except for one patient who became seropositive during the week after treatment; this patient then reverted to seronegativity. After 6 months, seronegativity was demonstrated in 81 per cent of the seropositive primary patients, in 65 per cent of the secondary patients, and in 73 per cent of the total. Only 4 patients required retreatment, but 3 of them had evidence of reinfection; the remaining patient had a sustained rise in the quantitative serologic titer. These retreatments provided a cumulative rate of 4.9 per cent.

The Smith report concludes: "Preliminary data indicate that a single injection of 2,500,000 units of Bicillin, in early infectious syphilis, produces results equal, if not superior, to those obtained with presently accepted treatment schedules."[2]

RESULTS IN ACUTE GONORRHEA

In the O'Brien study,[3] the therapeutic efficacy of a single injection of Bicillin was tested in three groups of male patients with acute gonorrheal urethritis. One group of 357 patients received 300,000 units; a group of 546 patients received 500,000 units; and a group of 125 patients received 2,500,000 units, a dose chosen because of its concurrent use in studies on syphilis.

In correlating the results of treatment with the original laboratory findings, O'Brien reports no proved failures. Of the 396 patients who returned for therapeutic evaluation after injection of either 300,000 or 500,000 units, 97 per cent experienced rapid and permanent subsidence of all symptoms. Since the outcome was unknown in certain other cases, O'Brien estimates a minimal cure rate of 95 per cent in patients receiving 300,000 units and 98 per cent in those receiving either 500,000 or 2,500,000 units.

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